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Dr. (Smt.) Muktha Sen, Professor of Maternity & Child Welfare, All-India Institute of Hygiene & Public Health, Calcutta.

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Sub-Committee on Protein Malnutrition

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Sub-Committee for Compiling Data on Physiological Norms of Indians

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Dr. B.K. Anand, Professor of Physiology, All-India Institute of Medical Sciences, New Delhi.

Dr. G.K. Karandikar, Professor of Pharmacology, Medical College, Baroda. (*Secretary*)

III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1957.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Mysore on the 26th and 27th November, 1956, and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 23rd March, 1957. *The views expressed by the individual workers are not necessarily the views of the Council.*

CLINICAL RESEARCH

1. Clinical Research Unit under Dr V. R. Khanolkar at the Indian Cancer Research Centre, Bombay.

1. *A Chemical method for estimation of chorionic gonadotrophic hormone (C.G.T.H.) in urine.*—The known biological methods for the estimation of C.G.T.H. are time-consuming and hence attempts are being made to develop a chemical method for their estimation. GTH being protein in nature, its quantitative estimation by means of the phenol reagent is being standardised. The details of the method are given in the report. The gonadotrophic activity of the hormone preparation obtained by this method is checked by a bioassay method. A mean concentration of GTH in male urine was found to be 6.11 ± 0.714 mg./24 hour (expressed as casein equivalent).

2. *Estimation of the non-protein nitrogen constituents of serum and urine in healthy subjects and in cancer with secondary liver and kidney dysfunction.*—A study on the non-protein nitrogen constituents of serum and urine in healthy subjects and in cancer cases with secondary liver and kidney dysfunction was undertaken. The purpose of the study was (a) to investigate whether the changes in serum and urinary levels of these constituents, as compared to those of healthy subjects, are characteristic of these diseases, and (b) to find out if any specific relationship existed in these diseases between the serum and urinary NPN constituents.

So far, 23 healthy individuals, 6 cancer cases with secondary liver dysfunction and 7 cancer cases with suspected kidney dysfunction have been studied for their serum and urinary NPN constituents. The six cancer cases with secondary liver dysfunction were characterised by the liver function tests. On the basis of the results of this study it appears that there is not an appreciable change in the serum NPN constituents in cancer cases with secondary liver dysfunction, but a high elevation in the same constituents is observed in cancer cases with suspected kidney dysfunction.

3. *Metabolic Studies on Thiosters of Hydrocarbons.*—Rats were fed with pure dibenzothiophene and their urine was collected. From the pooled urine, a colourless crystalline compound m.p. 257° (decomp.) and an oil have been obtained. Identification of these products is in progress.

2. Neurological Unit under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

1. Experimental observations on Lathyrism

In continuation of certain observations made last year further experiments were undertaken to test the hypothesis that the feeding of Lathyrus sativus could facilitate the noxious effects of neurotoxic agents in doses that would be innocuous to normal stock-diet fed animals. Four groups of young Wistar Rats including those maintained for a period of six months on either a 95 per cent or 100 per cent lathyrus diet and their respective controls, were challenged with graded doses of KCN administered as an aqueous solution by intraperitoneal injection, as follows :

Group (1) received 0·5 mg/kg. body weight, Group (2) 1·0 mg/kg., Group (3) 2·0 mg/kg. and (4) 3·0 mg/kg. body weight respectively per dose, by intraperitoneal injection. All the animals of Group (1) received a total of 8 injections (one per day), while the remaining three groups received 7 injections of the doses specified above. All the animals survived the full schedule of injections.

During periods ranging from one to three days after the last injection the animals were sacrified, and the brain proximal portion of the cervical spinal cord. and portions of the liver were removed carefully for histological examination. Histological changes of varying degrees were found in the brains of all the animals. Pyknosis, shrinkage of cellular outlines. tortuosity of the dendritic processes and homogenous deep staining, with loss of differentiation between nucleus and cytoplasm or pallor with progressive degrees of disintegration of the cell body, were the main nerve cell alterations. There was no appreciable difference in the pattern of distribution of such changes in Lathyrus-fed or control animals.

A rough quantitative evaluation of the changes observed in each of these animals was attempted, making this evaluation as objective as possible. The extent of the changes in both nerve cells and white matter was used as a criterion to indicate these in numerically increasing grades of N±, N+ etc. and W±, W+ etc. The sign + was given the value of 1, and ± of ½ respectively to both nerve cell and white matter changes. The total obtained for each of the experimental and control groups was averaged and expressed in the terms of average numerical values.

It was found (a) that there was no distinct linear relationship between the extent of the changes observed and the doses of cyanide

administered either in the experimental or control groups. (b) While th ... the Lathyrus-fed groups a ... re than their respective co ... ificant.

While the results obtained so far, are not considered to, in any way, significantly offer support to the hypothesis on which this experiment was based, it is proposed to conclude these investigations with observations on similar groups of animals treated with increasingly higher doses of cyanide.

II. Studies in Pyridoxine Deficiency

(a) *Monkeys*: One of the two monkeys—Monkey No. P-1 maintained on the dietary pyridoxine deficiency for the last 21 months, has been sacrificed for histology. At the end of this period, the animal had lost five pounds in weight, looked anaemic and highly emaciated with an extensive loss of hair all over the body, particularly over the joints of the legs. There was a marked fall in the levels of blood haemoglobin, vitamin B_6, plasma total protein, A/G ratio and iodine number of fatty acids, and a rise in the plasma alkaline phosphatase value. However, the levels of blood urea, plasma total lipids, and total cholesterol remained unaffected. These results appeared to be indicative of damage of the liver.

The histological examination of the viscera of this animal is in progress.

Monkey No. P-2 did not show any marked changes clinically. However, significant biochemical changes have been observed in the levels of vitamin B_6, A/G ratio, plasma alkaline phosphatase, and iodine number of fatty acids. Although direct correlation between Vitamin B_6 and iodine number of fatty acids could not be seen during the course of the induced deficiency, the prolonged deficiency brought about a marked reduction in the plasma iodine number of fatty acids after a period of 19 months, in both the monkeys. In the absence of adequate supply of pyridoxine it appears, the animals are unable to make proper use of essential fatty acids.

An increase in blood urea levels observed in pyridoxine deficient rats (Beaton *et al.* J.B.C., 200 ; 715, 1953) was not seen in monkeys.

(b) Rats: Experimental observations on induced pyridoxine deficiency in rats mentioned in our last year's report were continued.

Inbred, adult male Wistar Rats weighing between 150–200 gms. were used. The stock diet used is a modification of the diet employed by Tulpule and Patwardhan (I.J.M.R. 38 : 3, 1950) and Beaton *et al* (Arch. Biochem. Biophysics 51: 94, 1954).

The vitamin B_6 contents of blood and liver were determined by the microbiological assay of Atkin *et al* (Ind. Eng. Chem. Anal. Ed. 15,

141, 1943), liver and blood transaminase by that of Tonhazy *et al* (Arch. Biochem. 28 : 36, 1950), using Marsh *et al* technique for blood transaminase (J. Nutrition, 56 : 115, 1955). The estimation of urea was carried out by nesslerization using urease suspension, and the liver total lipids by the method of Channon *et al* (Biochem. J. 31 : 1736, 1937).

RESULTS :

(a) *Clinical.*—Early clinical manifestations of the deficiency such as roughness, patchy brown discolouration of the fur, reddening of the margins of the ear and skin of paws appeared in 1—2 weeks in both the pyridoxine deficient and deoxypyridoxine treated animals. There was however, a transient regression of these symptoms after 4—6 weeks. The severity of the affection of nose, paws and the anogenital region were observed at the end of 2½—3 months and 8—8½ months in the case of deoxypyridoxine treated, and pyridoxine deficient rats respectively. The delayed appearance of these symptoms may be attributed to the use of adult rats of 180—200 gms. This was necessary to have sufficient material for the entire biochemical studies. Using adult rats of same weight group, Ramalingaswamy and Sinclair, (J. Invest. Dermatology, 20 : 81, 1953) have observed that severe pyridoxine deficiency symptoms occurred after 6 months feeding of the rats on diet deficient in pyridoxine.

(b) *Biochemical.*—The effect of the induced deficiency by deoxypyridoxine appears to be similar to that of dietary pyridoxine deficiency so far as the levels of vitamin B_6 in blood and liver are concerned. While the levels of blood and liver transaminase diminish significantly in the case of deoxypyridoxine treated rats, only blood transminase values fall markedly whereas the liver transaminase is not significantly affected in the case of pyridoxine deficient animals. In one set of recovery experiments where the animals were kept for 3 months on deoxypyridoxine diet and then fed pyridoxine for 4—5 weeks (Group IV), the levels of vitamin B_6 transaminase and urea reverted almost to normal. The deoxypyridoxine fed rats showed two-fold increase in blood urea while these levels were only slightly elevated in the pyridoxine deficient group, as compared to controls. Definite correlation could be seen between the levels of blood and liver vitamin B_6 and transaminase in the control and experimental groups. The values of vitamin B_6 compare favourably with those of Tulpule and Williams (J. Nutrition, 57 : 529, 1955) and those of transaminase are higher than those reported by Caldwell and McHenry, (loc cit).

There appears to be no accumulation of fat in the livers of these animals and in fact the values of lipids in experimental groups are lower than those observed in controls. These results are in agreement with those of Caldwell and others.

Histological examination of tissues of the rats sacrificed so far is in progress.

III. Reference Work

1. *Kyasanur Forest Disease.*—During the course of this year the unit received specimens of brains and viscera from post-mortems performed on three fatal human cases of Kyasanur forest disease in Mysore, and similar specimens from about 10 monkeys found dead in the forest. Histological examination of these specimens have been completed and the findings are being prepared for publication

2. *Encephalitis material from Singapore.*—During the course of [illegible] from another 12, and incomplete specimens from 6 others. The first lot has been examined and has revealed histological findings of a disseminated non-suppurative encephalitis, suggestive of virus encephalitis in the majority of cases. Two of these cases revealed intranuclear eosinophilic inclusions. The rest of the material is under study.

3. *Miscellaneous.*—A total of 135 specimens, inclusive of muscle biopsies, neurosurgical biopsies, and brains obtained from post-mortems on neurological cases, were received for study

3. Clinical Research Unit under Dr. R.N. Chaudhuri at the School of Tropical Medicine, Calcutta

I. Clinical and Field Work.

A. *Tropical eosinophilia :*

A clinical study of 100 indoor cases.—Most of the patients were males (90) between the ages 11 and 30 and residents of Calcutta. Occupation and environment did not seem to have any influence on the incidence. A past history of allergic manifestations due to rhinitis, [illegible] cases, a few had [illegible] ird of the patients [illegible] r family members

Clinical features.—The onset was usually insidious, but acute in a few, the duration of illness was from 3 months to 23 years. The common presenting symptoms were cough and or bronchial spasm with or without expectoration. Other symptoms present in varying proportions were breathlessness, vague pains in chest, low irregular fever, loss of weight and occasional spitting of blood. Various non pulmonary symptoms and signs, mostly gastro-intestinal, were present but apparently unconnected with tropical eosinophilia. Infected throat was present in 25 per cent of cases. Rhonchi and rales were common findings in the lungs with emphysema in a few, while the main radiological changes were mottling and or increased bronchovascular shadows with hilar reactions.

Laboratory examinations.—Wbc per c. mm., average 22,470, highest 48,000, lowest 6,200. Eosinophils per c. mm., average 13,934 (62 per-cent), highest 35,360 (80 per cent), and lowest 2,088 (18 per cent). The common intestinal parasites were present in 21 per cent of patients. None had microfilaria in blood. Various other examinations were made, but the results were of no importance except that intradermal tests with dirofilaria immitis antigen were uniformly positive in all the 23 cases tested including 6 cases reported last year. In 5 cases the test was repeated after treatment with positive result. Eight control cases including 5 with microfilaria in blood gave negative results except one. With hydatid fluid it was positive in about half the cases, while with hookworm antigen obtained locally was uniformly negative. Biopsies of the liver, lymphnodes and skin showed nothing special except eosinophilic infiltrations. Spirochætes often found much in excess in bowel mucus and sputum, than in the control cases, were reduced after treatment. Blood of 104 family members of 31 patients were examined for eosinophilia of whom 11 had eosinophil above 20 per cent, maximum being 60 per cent in one.

In treatment, the preliminary report on diethylcarbamazine tried in 28 cases and submitted last year showed that with it the response was striking in 20 cases, the results being "indeterminate" in 5 and "failure" in 3. Of the failures 2 did not also respond to arsenic. The trial was carried out this year on a further series of 34 cases making a total of 62. The daily adult dose was 400-800 mgm. in divided doses for 5-7 days. The pulmonary symptoms usually disappeared within a week with a marked fall of eosinophil count in 2-5 weeks. Initial aggravation of cough and spasm occurred in 9 cases. Mild untoward reactions viz. nausea, pain in abdomen, slight fever. etc., were not uncommon, especially with larger doses, but did not necessitate discontinuance of the drug. Two cases however required a second course. The drug is quicker in action and efficient; next comes arsenical injections and after it carbarsone, aureomycin and antimonials. The last three are slow or unreliable. There was no advantage in combining diethylcarbamazine with carbarsone. Only 2 out of 5 cases treated with aureomycin, 0.5 gm., 6-hourly had complete relief. Prednisolone, as reported last year, had a temporary effect; it is very useful in cases with status asthmaticus. Antimony injections (I.V. or I.M.) were tried in 18 cases. They were not always tolerated well and the response was good in 6 of which one relapsed after a month. Spleen extract, sulphonamide, antihistaminics, urethane, penicillin and streptomycin were without effect.

The benign nature of the disease, long duration when untreated, relapses, absence of gross pathological changes and the fact that the condition is influenced by drugs with parasiticidal (helminthic) action and not by antihistaminics are in favour of an infection with one or more types of organism of low virulence. History of allergy in 27 per-cent cases and the usually prompt but temporary response to prednisolone suggest the possibility of an allergic factor.

A *follow-up* of 20 cases treated with diethylcarbamazine for a period of 3 to 12 months showed a further fall, on an average from 18 per cent

to 9 per cent. One relapsed after a year, but responded just as well as in the first attack.

(2) *Field Survey*.—In continuation of a preliminary survey on eosinophilia last year, in certain rural areas of West Bengal further surveys on the incidence of eosinophilia among 'normal' people in certain areas of Assam and West Bengal were carried out. In Goalpara district blood of 530 members of all age groups were examined both for absolute eosinophil and differential count. Eighty cases had eosinophil count above 20 per cent up to a maximum of 74 per cent, the highest incidence being noted in young age groups. Thirty-two cases had absolute count between 2,000—15,200 (2,000—3,000 in 24, 4,000—5,000 in 6, 5,300 in 1 and 15,200 in the other), but none of them showed any clinical manifestation. In Jalpaiguri district 628 subjects were examined. Night blood was collected for microfilaria as well as for differential count in the same subject. Eosinophil above 20 per cent was detected in 115, 21 having a count of 30 per cent and above up to a maximum of 64 per cent without any clinical manifestations. In 39 cases microfilaria was found in blood of whom only six had eosinophil count between 20 per cent and 30 per cent. These results do not indicate obvious association of high eosinophilia with filarial infection, although failure to demonstrate parasite does not of course exclude the infection. In a Factory near Calcutta where workers are exposed to gaseous substances and dust, 70 showed eosinophils above 20 per cent (20—30 per cent in 59, 31—50 per cent in 8 and 51—65 per cent in 3) out of 981 adult males examined. Two gave past history of asthmatic attacks. It indicates that exposure to vapours, dust etc., does not cause any increase of eosinophils nor aggravates pulmonary symptoms in cases who had eosinophils above 20 per cent.

Comments.—Three surveys are reported on the incidence of eosinophilia (20 per cent and above up to 74 per cent) among 2,139 apparently 'normal' people (men, women and children) in certain urban and rural areas of Assam and West Bengal including a factory area. The overall incidence of eosinophilia was 12 per cent and most of the cases were found in younger age groups (up to 20 years of age). The survey indicates that massive eosinophilia can exist without any symptom. In one area (Jalpaiguri) 6.5 per cent of the population examined were found to have microfilaria in their blood, but only 6 out of 39 positive cases had eosinophilia between 20 per cent and 30 per cent, the others had less.

B. *Tropical Splenomegaly*:

(1) *A follow-up study*.—Analysis of all the data of 290 cases of chronic splenomegaly showed that they were of varied aetiology as reported last year. Some of them were due to post hepatitis, portal cirrhosis, extra hepatic portal obstruction, thalassemia and metabolic disorders, but majority of them belonging to rural areas were sequel to chronic malaria, malnutrition being an associated factor, contributing to the hepatic changes which may lead to cirrhosis (aggravated by repeated antimony injections on mistaken diagnosis of kala-azar). A follow up study of 60 cases of tropical splenomegaly—43 treated medically and 14 after splenectomy, 3 having died soon after the operation was made, the total period of observation being 2 to 4 years.

In the non-operated series, 22 cases (4 years or less duration) in the 'early' stage were cured on suppressive anti-malarial therapy—weekly or fortnightly for half to one year. In the 'late' stage, 7 out of 14 patients (4—12 years' duration) there was remarkable reduction in the size of the spleen to 2″ or less from 6″—9″. Others remained in same state or became worse, one having died of hepatic failure following hæmatemesis. In the 'terminal' stage (8 to 18 years' duration), 3 out of 7 died and the remaining four had recurrent attacks of hæmatemesis. Seventeen cases in the late stage were subjected to splenectomy. Three died of operation, one of hæmatemesis and hepatic coma one year after and another from ascites and hepatic failure 2 years after while 5 were lost sight of. The remaining 7 patients complained of progressive weakness and lost weight by 4—15 pounds after the initial gain within one year although their blood picture was almost within the normal range. Two had hæmatemesis and melæna 1½ and 2½ years, after the operation. Irregular fever was complained of by 4 patients. Repeated needle biopsy of the liver showed necrosis of liver cells, cellular infiltration and fibrotic changes of varying grades in six of them, and the lesions were found to be more advanced than in the pre-operative biopsies. Careful assessment of a case especially with regard to the state of liver is therefore indicated before splenectomy.

It is of interest to note from the field study that as a result of anti-malarial measures for several years, the condition, hitherto a serious menace to public health in rural areas, has been virtually eliminated from the area.

(2) *Investigation for presence of abnormal hæmoglobin.*—Fœtal hæmoglobin was estimated in the Hæmatology Department in 32 cases, following the alkali denaturation technique of Singer *et al.* The average level of fœtal hæmoglobin was 2.12 per cent and the range varied from 0.0 to 4.4 per cent. Using an L.K.B. model paper electrophoresis equipment, electrophoretic analysis of hæmoglobin was done in 19 cases. All the patients showed a single spot having electrophoretic mobility of A hæmoglobin and the genotype in each case conformed to AA pattern. None showed any abnormal hæmoglobin.

C. *Spleen extracts.*—Acetone extract of bovine spleen was prepared in the laboratory and evaluated clinically in urticaria, bronchial asthma and tropical eosinophilia. Cases of urticaria showed variable improvement, a number of them being completely relieved during the period of observation for 3 months. Others had little or no improvement.

II. Experimental work

A. *Tropical splenomegaly.*—Continuation of the study showed that latent *P. berghei* infection in rat could cause chronic splenomegally of large size. Significant pathological changes in the liver, focal necrosis, cellular infiltration and fibroblastic proliferation, were also observed, and the fibroblastic activities were more marked if the diet was deficient. Initial deposition of malarial pigment in both the spleen and liver ultimately disappeared after 1½ to 2 years. The experimental findings

were thus somewhat similar to those in clinical cases. Control rats treated with mepacrine after each inoculation showed no significant splenic enlargement and hepatic involvement.

B. *Amœbiasis.*—Intra-cæcal inoculation of crude culture of *E. histolytica* in guinea pigs produced acute intestinal amœbiasis, leading to death within a week or so. Some changes in the liver were also observed. The diarrhœic manifestation was, however, conspicuous by its absence in all these animals and, therefore, it was not always possible to examine the stool. The cæcal content at the time of sacrifice showed, however, presence of amœba in most of them. To facilitate repeated examination of the cæcal content for *E. histolytica*, without sacrificing the animal or surgical measure, a method was devised by application of a permanent hollow metallic fixture into the cæcum for easy inoculation as well as withdrawal of fæcal material for examination, whenever required.

4 Inquiry on the Endemic Focus of Schistosomiasis under Dr. H.I. Jhala at the Grant Medical College, Bombay.

Infection experiments on Snails.—About 15,600 snails (Ferrissia tenuis) were infected during the year with different doses of miracidia. A majority were infected en-masse. 10,600 F. tenuis collected from nature at Gimvi were infected at Gimvi and were brought to the laboratory. 1200 laboratory bred snails were taken to Gimvi in an atmosphere of oxygen, infected at Gimvi and were brought back to the laboratory. Samples of urine obtained from patients in Bombay showed considerable fluctuation in the number of viable ova, and in the degree of activity of hatched out miracidia.

The infected Ferrissia tenuis that survived a period of 5 weeks after infection emitted few cercariae. A yield of limited number cercariae was available time and again. This however, was too less for animal infection. Due to this the infected animals were found to be negative after a period of three months.

It was observed that a majority of the infected snails die in the laboratory because of the infection and partly due to the failure of air compressor plant. F. tenuis are very small in size and quite delicate. It is also observed that these snails are not good host capable of prolific liberation cercariae. At Gimvi it is found that the rate of infection in ferrissia is around 2 per cent in general.

Indoplanorbis exhustus and a good number of Limnæa luteola were also infected in the laboratory with S. haematobium but majority of the snails did not survive the period of incubation. Those that survived were found negative on dissection.

Animal infection :—

(a) With S. haematobium :

Different methods of animal infection were tried on mice, young rabbits, but these gave negative results as the cercarial yield was very poor.

(b) With S. Spindalis :

Mice and rabbits were infected with cercariae emitted by naturally infected Indo-planorbis exhustus and Limnæa Luteola snails. A good number of S. Spindalis adult worms were recovered after three months of infection. The cercarial yeild from these snails was considerable.

Survey of the village Gimvi.—Survey of School children at Gimvi was carried out in the month of January, 1957. Out of 29 cases examined 18 were with viable ova (out of these six had gross haematuria), Seven with non-viable ova, and 4 were negative.

It was observed that the population of ferrissia tenuis was excellent where the structure of the river bed is stoney, water level is shallow, and when there is a constant movement of human beings and animals.

Experimental work.—Indo-planorbis exhustus were used in experiments on quantitative cercarial yield, and the effect of heat, light and salinity in different grades on the emission of cercarial.

Experiments were also carried out on phototrophicity of cereariæ, —viability of F. tenuis in different conditions of oxygen requirement in an atmosphere of oxygen.

Experiments on the viability of S. hæmatobium ova under different conditions of media and temperature totally failed. Staining, clearing and mounting of adult worms (S. spindalis) was done satisfactorily.

Re-Survey of village Gimvi. – House to house survey of Gimvi was carried out in first week of September 1957. In this survey people born, and brought up in the village, and no casual visitors were also taken into account. The total population worked out to be 933 out of which 468 were males and 465 females, 676 per male were staying in the village and 257 were casuals at the time of survey.

Infection was maximum in Lower-Marathawada. About 40 per-cent of males staying in Lower-Marathawada had been infected some time or the other. The incidence was maximum in the age group of 5—15 years, and the 5—25 years group was found to be the usual period of incidence.

It was observed that a majority of cases treated by the Public Health Department, Poona, in May, 1955 showed relapse. Three fresh

cases of infection were found in Lower-Marathawada and one in Upper-Marathawada.

A paper on the detailed survey work is in the press.

GENERAL

Foreign workers were consulted about certain aspects of the research work but no satisfactory reply was received from any of them. An invitation to attend the African Conference on Bilarsiasis held at Brazzaville on 26th November to 8th December, 1956 was received. An express letter was written to Director, Indian Council of Medical Research if the Council or W.H.O. would finance the deputation of one of the members of this enquiry. This was not arranged perhaps due to short notice.

A research scheme presented by Dr. A.K. Varma of the Live Stock Research Station, Bihar was well commented upon by us.

5. Inquiry into the use of artificial hypothermia (hibernation) in open intracardiac surgery under Dr. P.K. Sen at the Seth G.S. Medical College, Bombay.

Altogether ten experiments have been carried out since 1st August 1957. This work has been divided in two main heads.—

A. *Studies in extreme hypothermia.*—To carry out open 'dry' cardiotomy on completely non-pulsatile 'standstill' heart with a view to exclude cerebral anoxia, ventricular fibrillation and air embolism. A group of seven experiments, to study the behaviour of the heart at low temperatures ranging from 22°C. to 10°C., have been carried out.

B. A further group of three experiments was conducted to produce a partial cooling to about 32°C., and producing chemical cardiac arrest by perfusing the coronary circulation with potassium-citrate solution through the ascending aorta after outlet clamping.

A. EXPERIMENTAL PROCEDURES

Adult mongrel dogs were anaesthetized with intravenous pentothal (30 mg /kg. body-weight), an endotracheal tube was inserted and the lungs hyperventilated (18 times per minute). Venesection was done and intravenous drip started. Electrocardiographic control tracings were taken, and animals were immersed in ice-cooled water (at temperature 0 to 4°C.) after recording the original temperature with rectal electric thermo couple. The rectal temperature and ECG records were maintained every ten minutes. When the required lower temperatures were obtained the cold water was siphoned off, and replaced by tapwater. The temperature of bath was raised to 45°C by adding warm water to re-warm the animals to their original temperatures.

Out of the seven dogs, three went into complete cardiac arrest (Nos. 4, 7 and 15), and did not register any beat after re-warming.

Dog No. 5 when cooled to 10°C., registered 4 beats per minute. On re-warming, it developed ventricular fibrillation at 27°C., and died. Dogs Nos. 9, 12 and 17 registered less than 10 beats per minute when re-warming was started. There was full recovery of the heart beats, reflexes returned and automatic breathing started, but all died within three hours of revival.

B. Experimental procedure

Adult mongrel dogs were anæsthetized with intravenous pentothal (30 mg./kg. body weight), an endotracheal tube was inserted and ventilated at a rate of 16 per minute. Electrocardiographic control tracings were taken. The original temperature was recorded with rectal electric thermo couple and animal immersed in ice-cooled water. The temperature and E.C.G. were recorded every ten minutes till the temperature fell to 32°C. The animal was taken out of the bath and placed on the operation table. After drying, the animal, the chest was opened by a transverse incision at the level of 4th intercostal space. The sternum was cut across and retracted. The pericardium incised. The azygos vein was isolated and ligated. The superior vena cava and inferior vena cava were isolated and clamped, and lastly the ascending aorta was clamped. A solution of potassium citrate in blood was injected in the aorta, proximal to the clamp to perfuse the coronary arteries till the heart stopped in diastole. After four minutes of complete cardiac arrest, plain blood was injected into the aorta, proximal to the clamp, under pressure, to wash out the potassium citrate from the coronaries. The clamp was released. The heart started beating, the beats being weak initially and showing some fibrillary twitchings. Calcium chloride was injected in the aorta after applying the clamps again when the heart beats improved but were not well sustained.

In these three experiments different concentrations of potassium citrate (25 per cent, 10 per cent, 5 per cent) have been used, these being of the nature of early pilot procedures. The optimal concentration of the cardioplegic drug varies with many factors including that of temperature.

6. Effect of metaphyseal stimulation of longitudinal bone growth under Dr. B. Mukopadhya at the P.W. Medical College, Patna.

This series of experiments have been carried over from the year 1954 through 1955 to 1956 and 1957. On the basis of observations made in 1954 and 1955, it was felt that the failure to obtain increase in length of a growing bone could be explained on the basis of a comparatively transient increase in the vascularity of the region of the metaphysis produced by the implantation of thin soft autogenous grafts taken from

the same young animal. In order to make the process of increase in vascularity more sustained, hard cortical adult bone removed from normal healthy adult goats and preserved by chemical sterilization was used in the present series as the grafted material. It was thought that such grafts will take longer to be absorbed and will thus produce more prolonged increase in vascularity. The process of absorption of the graft was followed by serial X-rays and in order to avoid cessation of the vascular response repeated grafting was performed in the present series of experiments. The following observations were made :—

(1) Even repeated grafting and sustained increase in the vascularity of the diaphyseal side of the epiphyseal cartilage does not produce acceleration of the rate of proliferation of the cartilage columns and does not produce any permanent increase in the length of the growing bone.

(2) Repeated grafting very definitely produces sustained increase in the vascularity of all the layers of tissues, i.e. deep fascia, the muscle, the periostum of the region of the metaphysis of the long bones.

(3) Repeated grafting also produces an inflamatory response and this is accompanied and followed by replacement of fibrosis causing adhesion of different layers of tissues adjacent to the joint and thus may be a factor in ultimately producing rigidity of the joint.

(4) Repeated grafting appears to have caused varying degrees of direct injury to the epiphyseal growth plate leading to either retardation or cessation of growth in the cartilage plate.

(5) Repeated grafting is technically a difficult procedure and unless performed with extreme caution may lead to pathological fracture and thus cause a deformity of the limb.

It appears wrong to equate increase in the vascularity of the region of the metaphysis to a stimulating agent of the epiphyseal growth plate. The other point to note is that the metaphyseal side of the epiphyseal growth plate is not really the proliferating section of the growth plate and thus increase of vascularity of this area may not stimulate rapid multiplication of cells.

7. Electro-Retinography in Vascular Hypertension under Dr. R.P. Dhanda at the M.G.M. Medical College, Indore

The earliest stage of the work on this enquiry is to standardise the normal electro retinogram of Indians over the age of 40 years and study variations if any with the younger age group already reported from this E. R. G. Unit in the years 1952-53.

During period under report 20 normal eyes of patients over the age of 40 years were investigated only those cases were selected whose vision

was not poorer than 6/18, there was no lens opacity, fundi were normal except the usual senile changes expected in a person of this age group and the blood pressure was within normal limits (i.e. 140/90). It may be stated that it is not very easy to collect normal subjects fulfilling these conditions in the out-patient Department particularly over the age of 40 years. In each case the vision was recorded, ophthalmoscopic examination was done and blood pressure was noted.

The patient was dark adapted for 5 minutes and electro-retinograms were recorded on a Sanborn Cardiograph machine by the method already established at this E. R. G. unit at Indore. The stimulus of 1/4 seconds of an intensity of 80 lux and at a interval of 15 seconds was maintained in all cases in this investigation of normal Indians.

The average b-pot of 26 eyes of 22 normal Indians above the age of 40 years has been calculated to be ·29mV which is distinctly less by ·03mV when compared to ·32mV of the normal persons of the age group 15—40 years. This subnormal electro-retinogram in older age group may be due to the senile changes at this age.

The table on this page gives the details of the observations in each case:

Serial No.	*Name*	*Age*	*B. P.*	*b-pot in mV*
1	S.K.	40	106/70	R.E. ·4/7
2	B.R.	48	130/80	R.E. ·137/10
3	S.K.	40	138/90	R.E. ·4/10
4	B.R.	45	112/76	R.E. ·3/15
5	G.R.	46	126/88	R.E. ·25/12
6	M.K.	55	120/76	R.E. ·3/15 L.E. ·4/12
7	G.S.	50	132/76	R.E. ·3/12
8	B.R.	47	100/70	R.E. ·32/14
9	R B.	50	112/74	R.E. ·345/11
10	V.H.	53	124/80	R.E. ·30/8
11	M.G.	55	112/78	R.E. ·30/11
12	V.D.	50	130/86	R.E. ·250/6 L.E. ·222/9
13	B.L.	50	118/84	R.E. ·2/7 L.E. ·2/7
14	G.B.	50	110/70	R.E. ·111/9
15	S.L.	42	124/90	R.E. ·266/3
16	R.	52	110/66	R.E. ·2/6
17	J.B.	40	116/76	L.E. ·315/8
18	R.B.	50	120/84	R.E. ·411/13
19	V.P.	57	110/70	R.E. ·308/6 L.E. ·306/8
20	L.M.	65	140/70	L.E. ·255/16
21	R.C.	48	128/82	R.E. ·30
22	S.D.	55	118/82	R.E. ·302/9

This E. R. G. Unit has already established during the last 5 years that the electrical potential recorded from the normal retina of Indians *between the ages of 15 and 40 years is ·32mV.*

Stimulus to this work on the clinical application of electro retinography in vascular hypertension was provided by the experimental work by Henkes who reported that the *electro-retinogram was found to vary* with sudden lowering and raising of blood pressure in animals ... retina and kidneys ... rtension and there- ... ro-retinography the eyes of patients suffering from benign and malignant hypertension. This can easily be understood because the associated changes in blood vessels will affect the nutrition of retina which in turn will effect the function capacity of retina and ultimately altering the electrical potential produced at the level of the retina.

With this object in view of the work on ENQUIRY ON VASCULAR HYPERTENSION has been planned to be completed in the following stages :—

1. ... ians above the age ... those already re- ... E. R. G. unit.

2. Study the effects on the electro-retinogram of increasing the sensitivity of retina by varying the dark adaptation again in the normal Indians over the age of 40.

3. ... ro-retinogram in cases of ... nsion and correlate these ... oscopic findings and the

4. Study the effects of treatment on electro-retinogram and thus, if possible, to assess the prognostic value of electro-retinography in vascular hypertension.

8 Inquiry entitled "Biochemical and Serological investigations on Escherichia coli and Klebsiella strains isolated from urinary infections" under Dr. S.P. Gupta and Dr N.P. Gupta at the Medical College, Lucknow.

(1) Collection of strains.—1030 strains of coliform organisms isolated from established cases of urinary infection have been so far collected in this department. They have been preserved in one per cent agar stabs.

(2) *Biochemical reactions.*—So far 900 strains have been tested for their biochemical reactions with 27 media. The result indicate that on the basis of the pattern of biochemical tests, about 60 per cent were Escherichia coli

The use of KCN broth to differentiate Escherichia from Klebsiella was continued. Strains belonging to Escherichia coli did not grow in KCN broth in 24 hours while Klebsiella grew well in the medium. This test was however not found specific, as about 7 per cent of Klebsiella grew after 48 hours and about 21 per cent of Escherichia strains were found to grow in the medium in 24 hours.

(3) Hæmagglutination tests were continued with these organisms. 345 strains of Escherichia were tested with red cells of sheep, rabbit, rat, man, chicken and guinea pig. Of the 345 strains of Escherichia coli tested, 164 strains were found to cause agglutination of red cells from one or several animal species. The guinea pig cells were most frequently agglutinated, while sheep and rabbit cells were agglutinated by least number of strains. Red cells from rat, man and chicken occupied an intermediate position. Many strains agglutinated only one species of red cells. Human red cells alone were agglutinated by 34 strains, guinea pig cells alone by 21 strains, rat cells alone by 15 strains, and chicken cells alone by 12 strains. Six strains agglutinated rabbit cells only and 2 strains agglutinated only sheep cells. 5 strains were found to agglutinate all the six species of red cells tested. The remaining 69 strains gave variable reactions.

There was no correlation between the hæmagglutinating property and the serological type of Escherichia strains.

The hæmaggluting strains were tested in the living state for the presence of L-antigen with unabsorbed 9 'OL' sera. 179 hæmagglutination positive strains have been tested so far. 92 strains out of 179 did not show any agglutination with these sera. 87 strains could be typed and their L-antigen could be determined. Maximum number of strains were grouped under L-antigen 1.

(4) Test for hæmolysin were carried out with all the Escherichia strains and it was found that 58 strains possessed this property. There was no significant correlation between the hæmagglutinating property and capacity to produce hæmolysis except in 5 strains out of 449 strains of Escherichia which produced hæmolysis and agglutinated guinea pig red cells. Hæmolytic activity was not confined to any well defined serological group.

(5) *Serological studies on strains of Escherichia.*—(a) Determination of 0-group of Escherichia strains with the help of 25-0-sera (constituting the diagnostic schema of Kauffmann, Knipschild and Valene) after preparing the antigen at 100°C. for one hour and again at 120°C. for 2½ hours have been continued.

(d) Determination of L-antigen of Escherichia strains.

Sera against 15 L-antigen were prepared in rabbits and preserved in neutral glycerol. Determination of L-antigen has so far been carried out with unabsorbed 9 'OL' sera, 316 strains of Escherichia coli were tested for the presence of L-antigen in living state by slide agglutination

method. 127 strains out of 316 could not be typed with these nine sera. It was also noted that these strains did not belong to 25-O-groups constituting the diagnostic schema. 31 strains were auto agglutinable. Maximum number of strains possessed L-antigen 1 and L-antigen 15.

Thus 41.93 per cent strains of Escherichia coli could be indentified serologically with the available 9 'OL' sera for the presence of L-antigen. Tests with further sera are in progress.

9. **Investigation into the role of allergens and various other factors in the production of bronchial asthama in Rajasthan in general and in Jaipur area in particular under Dr R.M. Kasliwal at the S M.S. Medical College, Jaipur.**

The pollination calendar of a place is a record of the time in days and months when pollens capable of causing allergic manifestations are present in the atmosphere. It will be realized that the first step towards distinguishing local allergenic plants is to obtain a thorough knowledge of the local vegetation as well as of the time when they effloresce. It is also equally clear that the problem is as much botanical as medical. To accomplish this work a complete botanical survey of the area in and around Jaipur city has been done and the period when the vegetation effloresces has been recorded. The newer additions to the flora, investigated already, is given below :—

Trees.—Acacia arabica, Albizzia odoratissima, Phoenix sylvestris, Cassia marginata, Cassia siamea, Cassia surattensis, Cassia javanica, Eucalyptus tereticornis, Bauhinia tomentosa, Melia azedarach, Salix tetrasperma, Holoptelea integrifolia, Phyllanthus emblica, Polyalthia longifolia.

Weeds.—Cyperus rotundus, Amaranthus viridis, Bulbostylis barbatus, Cyperus compressus, Cyperus iria.

Grasses.—Dactyloctenium sindicum, Saccharum spontaneum, Cenchrus catharticus, Eleusine verticillata, Zea mays, Cenchrus biflorus, Panicum paspalodes, Imperata cylindrica.

The anemophilous pollens and the abundant entomophilous ones have been collected during the period of their pollination. A pollen library has thus been established. The pollen library has materially helped towards identifying the pollens on the catchment slides.

A daily census of the aerial pollen has been carried by the Gravity slide technique using the standard sampling device (Durham). An attempt was made to identify the individual pollens on the catchment slides. The following pollens were identified in the catchment slides :—

Trees.—Acacia, Callistemon, Leucaena glauca, Malvaceae, Morus alba, Dodonaea viscosa, Daucus carota, Mimosae, Holoptelea, Azadirachta, Albizzia, Delonix, Phyllanthus, Putranjiva, Leguminosae, Compositae, Cassia, Phoenix.

Weeds.—Amarantus-Chenopod, Argemone, Abutilon, Datura, Ricinus.

A correlation was done with the field survey and this double checking has resulted in pin-pointing the actual time in days and months when the pollination would be clinically significant from the point of diagnosing and treating an allergic individual.

The influence of the meteorologic conditions on the concentration of pollens as a whole has been studied. It was seen that the pollen count is effected by a number of factors, such as rainfall, humidity, temperature, duration of sunshine and the velocity of wind. A shower of rain tends to decrease the pollen counts, while a period of draught and the rise of temperature following a heavy rain is associated with increased pollen counts. Increased humidity seems to diminish pollen liberation and is associated with a decrease in pollen counts and vice versa. The rainy season in this part starts with the arrival of monsoon in the 4th week of June. The results of field survey showed that the vegetation effloresces from about mid of July but the catchment slides have shown that the maximum concentration in the atmosphere is attained some two months later because the rainy season persists during this time and the increased humidity interferes with the liberation of pollens.

To increase the allergenic flora prevalent here, mass clinical testing has been performed at an Allergy Research Clinic set up in the S.M.S. Hospital, Jaipur. All the patients of respiratory allergy are referred to at this clinic. With the help of a thorough history taking, clinical and laboratary investigations, an attempt is made to discover the offending allergen or allergens in these patients and then the I.C. skin tests are performed. So far 80 cases of respiratory allergy, including cases of asthma, allergic rhinitis, hay fever and allergic bronchitis have been investigated. Pollens of *Azadirachta indica* (Neem) ; *Amarantus spinosus* (Kateli chaulai ; (*Dodonaea viscosa; Holoptelia integrifolia* (chilli) ; *Phyllanethus emblica* (Amla) ; *Argemone mexicana* (Kateli) ; *Chenopodium album* (Bathuwa) ; *Ricinus communis* (Arand) ; *Chloris barbata*.; *Cynodon dactylon* (Dub) ; *Desmotachya bipinnata* (Dab) ; *Digitaria marginata* (Jada) ; *Elusine indica ; Pennisetum orientale ; Sorghum halepense* (Baru) have been considered to be definite offenders. A more definite information would, however, be available after testing a larger number of patients.

Of the 80 cases of allergy, 49 cases were found to be of pollen allergy, and hyposensitization treatment has been started in these cases. The success or failure of the treatment would take sometime to evaluate and more definite conclusion could be deferred till such time.

10. Inquiry on the formation, distribution, structure and development of the splanchnic nerves under Dr. Inderjit at the Medical College, Amritsar.

1. The origin, formation, distribution and termination of all the three splanchnic nerves have been followed on both sides in 94 instances

(47 cadavers). Fine branches have been followed with the aid of a dualoupe.

2. Similar dissection has been made in one full term foetus.

3. The histological structure of the nerve has been examined by making serial sections in the case of one full term foetus. Sections were stained with appropriate stains.

4. Complete serial sections of the two embryos have been prepared. Staining methods used include Masson's trichrome, H & E and de Castro. These will enable us to study the development of splanchnic nerves.

5. Detailed findings have been recorded on a specially prepared proforma. Photographs of important and unusual findings have also been taken.

11. Inquiry on the value of commercial silk grafts to bridge large blood vessel gaps under Dr. Yudhveer Sachdeva at the Medical College, Amritsar.

Transplantation of preserved aortic homografts in the abdominal aorta below the origin of renal arteries has been performed in 30 dogs. Grafts were taken from abdominal and thoracic aorta of dogs and were preserved in the following media :—

		Dogs
(i)	4 per cent neutral formalin solution at room temperature	10
(ii)	70 per cent ethyl alcohol at room temperature	10
(iii)	Balanced salt solution containing 10 per cent homologous serum and antimicrobial drugs at a temperature 2°C. to 6°C.	10

Neutral formalin solution was prepared by adding an excess of calcium carbonate to a 4 per cent solution of formalin and then filtering it. Grafts were preserved in this solution at room temperature. Seventy per cent ethyl alcohol was prepared by diluting absolute alcohol and grafts were preserved at room temperature. Grafts to be preserved in balanced salt solution were taken aseptically and the solution was cultured for the presence of pathogenic organisms before transplantation.

Formalin preserved grafts

In the first group, aortic homografts preserved in 4 per cent neutral formalin solution were used. In all 10 such grafts were studied in dogs weighing from 6.4 kg. to 11.0 kg. aortic segments were preserved from 6 to 28 days. The animals were sacrificed at varying intervals and changes in the graft studied.

Results.—In four animals post mortem was done 5, 11, 55 and 59 days after the operation (grafts were preserved for 19, 14. 11 and 11 days, respectively). There was no complication and the grafts were functioning well. The graft in the dog sacrificed after 55 days showed a few small thrombii.

320 days after transplantation of the graft, one of the dogs with 28 days preserved grafts is still under observation. It has good femoral pulsation.

Two dogs died of late hæmorrhage due to dehiscence of the anastomosis. One died after 4 days and the other after 7 days. Grafts transplanted were preserved for 14 and 9 days, respectively.

Two dogs developed paraplegia 2 days and 3 days after operation. Grafts were preserved for six days and 17 days, respectively. On post mortem the aorta was found to be occluded by a thrombus in each case.

In one dog femoral pulsation was absent but there was no paraplegia. Post mortem was done after 52 days of transplantation and the graft was completely occluded by two thrombii at the two ends of the graft (Graft was preserved for 14 days).

II. *Alcohol preserved grafts.*—Ten grafts were used in dogs weighing from 6·4 kg. to 13·6 kg. Time of preservation of the grafts varied from 2 to 48 days.

Results.—In three dogs post mortem was done 10, 32 and 44 days after the operation. Grafts were preserved for 1, 13 and 16 days, respectively. Grafts were found to be patent. Two dogs with grafts preserved for 3 days and 7 days are still under observation (178 and 147 days after transplantation of the graft). In one dog post mortem was performed after 82 days of operation (39 days preserved graft was used). There was a thrombus occluding the aortic lumen. There was no paraplegia. One dog died of hæmorrhage 5 days after operation due to dehiscence of the anastomosis (48 days preserved graft being used). Three dogs developed paraplegia and post mortem was done after 2, 14 and 19 days after transplantation. (Grafts used were preserved for 2, 15 and 10 days, respectively). On post mortem all the three grafts were found to be completely thrombosed.

III. *Grafts preserved in balanced salt solution.*—Ten such grafts were transplanted in dogs weighing from 6·4 kg. to 13·6 kg. Grafts were preserved from 1 to 35 days.

Results.—Five dogs are still under observation after 63, 31, 20, 15 and 12 days after transplantation of the grafts. Grafts were preserved for 3. 6, 4 and 5 days respectively. In one dog post mortem was done 47 days after operation (5 days old graft was used). The animal had no paraplegia, but a thrombus was found occluding the aortic lumen. In another dog post mortem was done 4 days after the operation (2 days preserved graft was used). The graft was patent. Two dogs died of hæmorrhage due to dehiscence of the anastomosis

2 days and 3 days after transplantation of the graft. Grafts were preserved for 13 days and 27 days, respectively. Two dogs developed paraplegia, 3 and 11 days, respectively after operation. Grafts preserved for 10 and 2 days, respectively, were used. On post mortem aortic lumen was found to be occluded by a thrombus.

Commercial silk grafts.—Tubes made of Kashmir silk have been used in 4 dogs. The tubes were made in such a way that the inner surface was smooth and the ends were everted to prevent fraying of the threads. The tubes were autoclaved. For some time before transplantation the tubes were soaked in the blood of the animal being operated and allowed to dry.

Results.—One dog died of hæmorrhage due to dehiscence of the anastomosis after 5 days of transplantation. Three dogs are still under observation after 73, 6 and 2 days of the transplantation of the prosthesis.

12. Study of renal changes following ureteric ligation and estimation of recovery following release of obstruction by ureteric transplantation under Dr. B.N. Balkrishna Rao at the G.R. Medical College, Gwalior.

Technique of study.—1. Incomplete obstruction is being produced by introduction of Laminaria tent 2.0 cm. long, through the left ureteral orifice in the bladder after opening the bladder. After various duration of time it is contemplated to perform nephrectomy on the right side accompanied by release of obstruction. Before release of tent, a retrograde pyelographic record to be kept

2. Sudden, complete obstruction in the ureter is produced by surgical ligation of left ureter at the level of the lower pole of left kidney.

3. Work on production of so-called idiopathic hydronephrosis is still in the preliminary stages.

4. The lymphatics of normal and hydronephrotic kidney and ureter is being visualized by injection of 1 c.c. of 11 per cent of patent blue into the wall of the ureter. A graphic record of lymphatics seen as distended blue streaks is being kept. This is being done to elucidate (if any) the role of lymphatics in absorption of hydronephrotic sac contents have been tubulovenous and pyelovenous backflow. Both have been observed during this inquiry.

5. The electrophoretic pattern of serum at various stages is being done. The densitometric and photographic record of strips obtained, are being maintained.

6. The kidney function is studied by injecting either 10 c.c. of 0·4 per cent indigocarmine of 6 mg. (1 c.c.) of phenolsulphonpthalein (P.S.P.)

7. Blood urea estimation is being done using King's technique.

8. Photographic records are being maintained at each stage of both kidneys.

DISCUSSION

It has been recorded from the work of the last two years, that restitution to nearly normal of hydronephrotic kidney of certain duration is possible. The recognition of this fact answers to some extent the queries usually posed :—

(i) For how long an obstructive lesion of the ureter, e.g. a calculus, which is causing complete or incomplete obstruction, be treated in a conservative manner ?

(ii) In what cases recovery of a hydronephrotic kidney is possible.

Our results so far differ fundamentally from previously published reports of restitution to normal of hydronephrotic kidney.

The knowledge gained through experimental work so far, has following important therapeutic applications :—

(i) Conservative treatment of ureteral obstruction may be followed without any increased risk for much longer than is believed at present.

(ii) The indications for nephrectomy need not be so frequent in cases of obstructive hydronephrosis. It should only be performed if sufficient time has clapsed after removal of the obstruction to allow the renal parenchyma optimum facilities for recovery.

The production of hydronephrosis by incomplete obstruction has produced many practical difficulties. The use of a Laminaria tent in the ureter, at least has the merit of producing a gradual obstruction, though incomplete. This state closely, if not accurately, simulates obstruction by a calculus which is growing in the ureter.

13. Inquiry into pneumoconiosis in the non-industrial population of Rajasthan with special reference to emphysema under Dr. R.K. Goyal at the S.M.S. Medical College, Jaipur.

The occurence of early pneumoconiosis in the apparently normal individuals of Rajasthan has already been reported to the I.C.M.R. These lesions in the non-industrial population can be found only in the desert regions. The lungs of apparently normal rabbits, guinea-pigs

nd white rats in Rajasthan have not shown the existence of pneumoco-iosis. Interstitial pneumonitis can be due to infective causes alone, hence he presence of this lesion exclusively is not important from the point f view of diagnosis. Granulomatous lesions associated with the pre-ence of silica particles are much more diagnostic. A histopathological xamination of the lungs of other uninjected animals which could be sed for laboratory work gave the following information :—

Three monkeys caught fresh from the jungle showed the presence f granulomatous lesions varying in extent

One of these monkeys was emaciated, a number of greyish-white laques were found in the lungs on naked-eye examination

On microscopic examination, definite granulomatous lesions ontaining giant cells and dust cells were present

Focal emphysema as well as thickening of some of the branches of he pulmonary artery were also found

An examination of the lungs of twelve stock dogs showed varying legrees of granulomatous lesions in eight out of them

Four dogs did not show definite evidence of pneumoconiosis, the esions, were slight or absent in the dogs aged 7 to 8 months

Out of the four goats examined, the one aged 7 months did not how any granulomatous lesions, the others aged 1 to 4 years had a few lust cells or dust-particles in the alveolar walls, and varying degrees of nterstitial pneumonitis were present in all these cases

Seven stock sheep aged 1 to 2 years were also examined

In addition to the presence of different degrees of interstitial neumonitis, four had dust cells or dust particles in the alveolar walls.

In a preliminary experiment, whilst the lungs of a pig aged 7 to months showed the presence of granulomatous lesions plus a few dust ells in the alveolar walls, the lungs of another pig aged 2 to 2½ months ld showed slight granulomatous lesions, but no dust particles in the lveolar walls It can, therefore, be concluded that monkeys, dogs and erhaps pigs, are very susceptible to pneumoconiosis Sheep and goats re less susceptible, whereas white rats, rabbits and guinea-pigs are ather resistant.

Inhalation experiments—A preliminary set of experiments had to e put up to determine the best method of producing pneumoconiosis n the experimental animals, and the results were as follows —

Rabbits—Five rabbits were made to inhale dust every day for 4 to hours in the inhalation chamber for 9 to 22 days

One rabbit which inhaled dust for 9 days continuously and was sacrificed 12 days after the commencement of the experiment did not develop granulomatous lesions

The other rabbits which were made to inhale the dust discontinuously with long intervals of rest and were sacrificed from 5 weeks to 5 months from the date of commencement of the experiment, developed lesions of early pneumoconiosis; three of them showed thickening and narrowing of arteriolar walls.

Guinea-pigs.—When one guinea-pig was made to inhale dust on alternate days for 10 days in all, and was sacrificed one month after the commencement of the experiment, no granulomatous lesions were discernible. When the inhalation was repeated in two other guinea-pigs after an interval of three weeks for a second period of 10 days, and the animals were sacrificed 3 to 5 months after the commencement of the experiment, granulomatous lesions varying in degree with a perivascular and sub-endothelial proliferation of connective tissue in a number of pulmonary arterioles were seen.

Rats.—The dust inhalation was either given daily or on alternate days for total periods varying from 6 to 64 days. The animals were sacrificed from 8 days after the commencement of the inhalation up to period of 7 months. Out of the 18 rats exposed, nine had slight lesions, five had moderate lesions and four had no granulomatous lesions. The results were rather irregular, the extent and type of the lesions could not always be correlated with the total period of the dust inhalation.

Monkeys.—Eight monkeys were exposed to inhalation of dust for periods varying from 10 to 38 days in all, they were sacrificed 13 days to 5 months after the commencement of the experiment. All of them showed granulomatous lesions varying in extent and containing dust cells, a definite thickening of the walls of some branches of the pulmonary artery was seen in four cases.

Two baby monkeys born in the Pathology Department were exposed in the inhalation-chamber for 7 and 11 days, respectively.

The first one was sacrificed after 2¼ months and the other after 3½ months.

The first monkey had very slight lesions, and the second had definite granulomatous lesions with focal emphysema.

These experiments were carried out to produce fairly marked lesions, as the preliminary experiments had shown that apparently normal monkeys in Rajasthan suffer from early pneumoconiosis. It appears that there is another factor responsible for the presence of marked lesions, this factor might possibly be an infective one.

The control experiments in which charcoal and calcium sulphate, etc., are to be used for inhalation are in progress.

Size of quartz particles.—A microscopic examination of human lungs carried out with the help of chemical polaroids revealed the presence of doubly refractile quartz particles at places where an aggregation of carbon particles was present. The size of the quartz particles did not

exceed 5μ. In the case of lungs of monkeys, most of the quartz particles did not exceed 5μ, but particles of 10μ in length were occasionally encountered.

The ordinary atmospheric air in Rajasthan does not cause pneumoconiosis in rabbits, guinea-pigs and white rats. When these animals are exposed to artificial dust-storms intensively in the inhalation-chamber, then alone they develop early lesions.

It is, therefore, apparent that dust storms constitute an important factor in the causation of pneumoconiosis. The microscopic examination of dust removed from the tops of almirahs showed the presence of a high percentage of particles which were less than 5μ in diameter, but all of them did not have the refractive index of quartz. The minute particles had also mostly lost their crystalline structure.

They constituted roughly 10 to 30 per cent of the dust in different samples.

14. Investigations of certain Ayurvedic preparations in treatment of Diabetes Mellitus under Dr. R. V. Sathe, at the Grant Medical College and Sir J. J. Group of Hospitals, Bombay.

The investigations in the use of Ayurvedic drug "Jasad Bhasma" (Zinc) were being done in the J. J. Hospital since April 1955, under the Indian Council of Medical Research Scheme. The procedure of [illegible] the year 1955-56. [illegible] n the last year's [illegible] The rest of the [illegible] The remaining 13 are cases treated during the year of report and are under observation for nearly one year. Much of the clinical material investigated has been wasted, as the diabetic patients come irregularly for a follow up. Out of 21 cases which are thoroughly followed-up during the year under report [illegible] ering [illegible] lica- [illegible] and the dose varied from 16 to 104 units a day. They were also on restricted diet—not taking sugar, rice and potatoes—but it was not properly calculated. The age group of the patients, when they came for treatment was: five were under forty years, while sixteen were above forty one years. The age of onset of the disease on the other hand showed that 8 persons had their onset below the age of 40; while 13 had it after 41 years.

THE DURATION OF DIABETES

Under	1 year (recent onset)		3
Between	1 to 5 years	...	14
Between	6 to 10 years	..	2
Above 10 years		.	2
			21

The diabetic cases can be divided, clinically into three groups :

(1) Mild—3 (2) Moderately severe—5 (3) Severe—13 out of these, 9 had complications as mentioned above.

THE RESULTS OF THE TREATMENT

These results with "Jasad Bhasma" can be summarized as under :

(1) *Urine Volume.*—The 24 hour urine excretion which was above normal, came to within normal limits, as reported in the last year's report.

(2) *Urine Sugar.*—The 24 hour urine sugar before treatment with Jasad Bhasma, varied from trace to 218 gms. a day. After treatment with Jasad Bhasma, it was reduced to nil in 13 cases, varied between 6 to 8 gms. in 5 cases, between 11 to 15 gms. in 2 cases and to 18 gms. a day in one case.

(3) *Blood Sugar Level.*—The blood sugar levels of all cases under treatment with Jasad Bhasma were lower than before as can be seen from the following table. For convenience cases under observation over one year are grouped as A, while those under observation for one year or less are grouped as B. Only fasting blood sugar levels are given below for convenience.

Fasting Blood Sugar	Before Treatment			After Treatment				
Level.	*Group A.*		*Group B.*	*Group A.*		*Group B.*		
80 to 120 mg%	Nil		Nil	3	+	2	=	5
120 to 160 mg%	1		2	2	+	6	=	8
161 to 200 mg%	2		3	2	+	3	=	5
201 and above.	5		8	1	+	2	=	3
Total	8	+	13					21

It will be observed that in eight cases which have been followed up from the last year's report (Group A) there was relapse of diabetic condition in two cases, due to no proper control, and in one case due to tuberculosis. The remaining cases continued to be better than before. In Group B, except two cases all showed definite improvement in the carbohydrate tolerance, after treatment with Jasad Bhasma. One case with diabetes and tuberculosis showed improvement very well, being aglycosuric with fasting blood sugar at 125 mg. per cent, without insulin. The original fasting blood sugar in this patient before Jasad Bhasma treatment was started was 315 mg. per cent and the 24 hour urine sugar excretion was 60 gms. (Case No. XV).

BLOOD UREA BLOOD CHOLESTEROL

The blood urea and blood cholesterol readings were found to be within normal range throughout the period of observation under Jasad

Bhasma treatment in all cases. No adverse effects on urea and cholesterol levels in blood were observed when the diabetic patients were under Jasad Bhasma treatment.

Haematologic studies

There is appreciably little change in hæmatologic studies except the reduction of hemoglobin values in some cases. Dietary inadequacy in the class of patients who attend the hospital may be one of the causes of subclinical findings in hemoglobin levels.

Weight

[illegible] l of observation for over one year, five [illegible] lbs; and three lost by 4 to 10 lbs. [illegible] of observation seven added weight by [illegible] to 14 lbs and five lost by [illegible] to [illegible] lbs, and one remained stationery. It will be seen therefore that out of the 21 cases reported 12 i.e. 57.0 % added weight and 8 i.e. 38 per cent lost weight.

Insulin

Before treatment with Jasad Bhasma six patients in Group-A were taking insulin and the dose varied from 10 to 104 units a day. After treatment with Jasad Bhasma only two cases required the help of insulin as they were of young age group.

In Group B, seven patients, out of thirteen were taking insulin, the dose varied from 24 to 80 units. After treatment with "Jasad Bhasma", out of these seven cases, six did not require the help of insulin, while one did not react to Jasad Bhasma treatment. Out of the 21 cases reported three did not react to Jasad Bhasma treatment and so required the help of insulin. Sugar excretion in 24 hours, with "Jasad Bhasma" and without insulin in 18 cases was as under :—

Sugar excretion in 24 hours - Nil in 12.
,, ,, ,, Trace to 10 gms in 4.
,, ,, ,, 11 to 20 gms in 2.

Six cases treated with Jasad Bhasma only without insulin at any time showed definite reduction in blood sugar level and urine sugar excretion.

Diet and Jasad Bhasma

This point was discussed in last year's report. Diet alone had not shown appreciable change in blood sugar level in some diabetic cases. This year two cases are discussed to illustrate the point. It was observed in a control case that even though the patient was kept on a diabetic diet (Case No. 17) for 27 days his fasting blood sugar level was lowered from 231 mg. per cent to 202 mg. per cent only. So diet for 27 days did not show appreciable change in lowering of blood sugar level.

Another patient of severe diabetes (Case No. 20) with fasting blood sugar level of 300 mg. per cent was kept on diabetic diet for 21 days without any other treatment. Her fasting blood sugar level lowered down to 280 mg. per cent—a difference of 20 mg. per cent only. The patient was kept on the same diet and "Jasad Bhasma" was started. After six weeks of "Jasad Bhasma" treatment the fasting blood sugar level lowered down from 280 mg. per cent to 180 mg. per cent a difference of 100 mg. per cent. The patient added 4 lbs., in her weight also. This showed that "Jasad Bhasma" had a definite beneficial effect on carbohydrate tolerance.

Whenever Jasad Bhasma was stopped and the patient remained on the same diet, increase in blood sugar level was observed after some variable period. This showed definitely that Jasad Bhasma in some way helped carbohydrate utilization.

THE PLACE OF "JASAD BHASMA" IN THE TREATMENT OF DIABETES MELLITUS.

It has been observed that "Jasad Bhasma" acts better in maturity onset type of diabetic patients. Patients with onset of diabetes in young age group are less amenable to oral therapy, and more liable to relapse.

The effect of Jasad Bhasma on the diabetic syndrome was observed usually after 4 to 6 weeks. It varied with the individual case ; depending upon the severity of the disease, accompanying complications and also the age group of the patient. Earlier the diabetes was detected and properly treated better the results. Uncomplicated cases reacted much earlier and in most satisfactory manner. Infection and irregularity in diet upset the carbohydrate metabolism, but Jasad Bhasma treatment improved the same.

Case No. XV - M.P.—A patient of severe diabetes with tuberculosis is worth mentioning. This case required 80 units of insulin a day. The intial blood sugar level here was 315 mgm. per cent. With Jasad Bhasma for four months she became aglycosuric and the fasting blood sugar came down to 125 mg. per cent.

It has been observed that some cases did not react to Jasad Bhasma treatment, and no definite cause could be ascribed for this. Diabetes is not a very simple problem and no definite cause for insulin inadequacy can be ascertained in each and every case. But it is worth while studying the effect of these minerals in Ayurvedic form, Jasad Bhasma, etc., as their cost is much less and no side effects have been observed thus for in cases treated for nearly two years. Another aspect of this treatment is that even though the drug takes a long time to act—it does not act immediately and rapidly like sulfonyl-urea compounds—the effects are long lasting. This can be seen from the case reports given in the appendix.

15. Inquiry on the metabolic fate of gelatin administered as a plasma substitute under Dr. C. Sivaraman, Senior Scientific Officer, National Chemical Laboratory, Poona.

Preliminary experiments were carried out to study the effects of transfusion gelatin of known average molecular weight on red cell, Hb and plasma protein concentrations, W.B.C. count, hæmatocrit erythrocyte count, sedimentation rate and coagulation time in adult albino rats maintained on the laboratory stock diet. The efficiency of gelatin in the treatment of shock was also determined on the basis of the survival rate of rats. Experiments were carried out with dextran using albino rats as experimental animals. The results obtained were identical with those obtained with gelatin in that the hæmodilution caused by dextran infusions resulted in a decrease in erythrocyte count, hæmatocrit, and Hb and plasma protein concentrations. With dextran administration, however, the sedimentation rate and coagulation time were slightly higher than those obtai [illegible]
allergic reactions were [illegible]
shock as determined by [illegible]
blood volume and transfused with an equal volume of dextran was found to be comparatively lower than with gelatin. In a few instances, dextran was thought to be the immediate cause of death due to anaphylactic shock although evidence for this is not conclusive.

The physico-chemical studies were carried out for purposes of characterization of the transfusion gelatin prepared on a pilot plant scale.

The next step in the investigation was directed towards testing this material for pyrogenicity, toxicity and allergic and antigenic reactions.

All animals tolerated parenteral administration of gelatin and manifested no alterations in their physiological well being. All animals gained weight and showed no anorexia.

Nutritional Evaluation.—The next phase of the investigation was designed to determine whether intravenous gelatin could be anabolically utilized. The studies were conducted on two groups of albino rats maintained on a low protein vegetarian diet containing 5% rice protein. After depleting the protein stores of the animals by maintenance on the diet for two weeks, one group of animals received, in addition to the diet 2 c.c. of gelatin intravenously twice a week for three weeks, the control group receiving by vein normal saline in place of gelatin. Experiments were now in progress to determine whether transfusion gelatin supplemented with amino acids administered orally as well as intravenously would promote utilization of the transfusion material for protein synthesis.

Results of Retention Tests.—These experiments were carried out on doubly depleted dogs and rats and on normal rabbits. Gelatin concentration in blood and in urine was determined according to the method of Jacobson and Smyth (Arch. Int. Med., 74, p 254, 1944). Hæmatocrit

was determined after centrifuging the heparinized blood samples in Wintrobe's tubes for 30 minutes at 3,000 r.p.m. Blood volume was estimated by injection of T—1824 Evans blue dye according to the method of Metcoff. Cutting and Favour (*Amer. Jour. Physiol.*, **141**, p.695, 1944). Nembutal was used as the anaesthetic at a level of 30 mg/kg. body-weight.

Histopathological Study.—Opinion is divided as to whether plasma expanders, such as dextran, polyvinylpyrrolidone (PVP), gelatin etc. cause anatomic changes in the internal organs. This aspect of the problem is of considerable importance in connection with the use of gelatin for transfusion purposes. However, anatomic changes were conspicuous in one specimen only. The structural alterations appear to be similar to the transient changes in the kidney reported by Popper *et al* (1945) following gelatin administration. To elucidate this point, further experiments are in progress. Histochemical tests with biopsy specimens (Morehead and Little, 1945) were all negative indicating that there was no deposition of gelatin in the tissues.

16. Development of a simple method of preserving or preparing aortic and arterial homografts under Dr. Timothy Takaro and Dr. A.C. Fletcher at the Wanless Tuberculosis Sanatorium, Wanlesswadi.

The experimental method consisted of the implantation of grafts 3 cm. to 5 cm. in length in the abdominal aortas of mongrel dogs. The graft material consisted of (*a*) homografts fixed and preserved in formalin, (*b*) grafts made of flexible, corrugated braided nylon tubing, (*c*) homografts fixed and preserved in 70 per cent ethyl alcohol.

Fifteen dogs each have been used for formalin-fixed and nylon grafts, and five dogs for alcohol-fixed grafts. Formalin-fixed grafts are technically satisfactory to handle and early results have been good. However, when animals were sacrificed 5 to 12 months after operation most of the grafts exhibited calcification and degenerative changes. The nylon grafts were technically more difficult to handle, but the late results have been superior. The grafts have remained patent and the lumen is found lined with a layer of intima.

Alcohol-preserved homografts have been reported upon by Kimoto, *et al.* (1954) and by More and Kajikuri (1956). This technique has been adopted recently and only five dogs have been operated upon. There were two early deaths, due to rupture of the graft, possibly a result of improper preservation. The remaining three animals have not yet been sacrificed.

The use of homografts, preserved in formalin or in alcohol, has the advantage over nylon grafts, that complicated segments, such as the arch of the aorta, are easily prepared and kept ready for use when needed. It seems likely that alcohol may prove preferable to formalin as a preserving agent.

17. Inquiry on bio-microscopical study of the conjunctival vessels in relation to the general arteriosclerosis and coronary artery disease under Dr. K.N. Mathur, Dr. K.S. Mathur and Dr. P.N. Wahi at the S.N. Medical College, Agra.

A total number of 215 cases were examined of which 80 were normal cases, 95 were Hypertensive cases and 40 coronary disease cases.

Biomicroscopical study of the conjunctival vessels was done by the technique perfected last year. Eighty normal, 95 hypertensive and 40 coronary diseases cases were examined.

Ninety-five of hypertension were studied. It was found that in hypertensive cases with the increase of blood pressure, the elasticity of the conjunctival blood vessels became progressively lesser, and abnormal tortuosity, loops, sharp bends, aneurysms and irregularities in the vessel calibre tend to increase. The lessening of the elasticity of the conjunctival vessels was manifested by the lesser extent of the maximum dilatation and in taking more time to reach the maximum dilatation, and in coming back to normal. The duration of maximum dilatation was also greater.

It was noted that in some hypertensive cases the maximum dilatation occurred comparable to that attained in the normal cases of the same age group, but time taken to return to normal was more while in other hypertensive cases though the extent of dilatation was less the time taken to return to normal had not altered.

Forty coronary disease cases were examined. All of these were confirmed by electro-cardiogram. The elasticity of the vessels had no direct relation with the coronary disease. It was only indirectly related, depending upon the blood pressure of the case.

In 27 cases of hypertension blood cholesterol was determined, and it was found elevated in 21 cases only. Blood pressure of these cases ranged between 150 mm. and 230 mm. of Hg systolic and 85 mm. and 100 mm. diastolic. All the cases showed decrease in the elasticity of conjunctival vessels, according to the range of hypertension. Blood cholesterol had no direct relationship with the elasticity.

In all cases of hypertension fundus examination was done under homatropine. Fundus findings were positive in 54 per cent of the cases. Lowering of the conjunctival vessels elasticity was found in all cases whether they had any fundus finding or not.

18. Inquiry on histo-pathological study of trachoma in relation to the clinical stages of the diseases under Dr. Pashpati Nath Wahi, and Dr. P.N. Awasthi, S.N. Medical College, Agra.

During the last and the present year 83 biopsies have been registered out of 105 cases of trachoma. The control cases so far studied are so few in number that it will not be possible to comment upon

Clinical Material.—The clinical material is provided by the In-patient and Out-patient Departments of the S.N. Hospital, Agra. The medical students also volunteered to act as control cases. Six groups of cases are being studied, based on the 4 clinical stages of Maccallan.

Biopsies.—The biopsies are taken both from upper and lower fornices after infiltrating the lids with 2 per cent novo-caine. A stitch is passed just above the lid margin and the lid so is everted. A chalazion forcep is applied on the everted lid so as to include the tarsal plate. A vertical incision is given on the tarsal plate and an incised piece of tarsal plate including conjunctival epithelium is removed and fixed in 10 per cent formal saline. The biopsy piece is processed and cut and stained with haematoxyline and eosine.

Observations and results.—172 cases have been registered up to 30th September, 1957. They comprised 10 asymptomatic cases and 158 cases of trachoma, 3 cases of chalazion and one case of spring catarrh. The age of the patients ranged from 10 to 70 years. The clinical diagnosis did not give much difficulty in well established cases. Asymptomatic and early cases presented difficulties which were finally labelled by clinicopathological data. Most of the cases came from poor and middle class, only one percent of cases were from higher class.

Histopathological diagnosis in 168 cases in various clinical stages has been done.

Smear examination findings.— As described by Linder, we came across two types of inclusion bodies; (1) initial bodies, and (2) elementary bodies. Initial bodies were seen in 66 per cent of total cases so far registered, whereas elementary bodies were noted in few cases.

Therapeutic response of various drugs in trachoma.—62 cases of trachoma in various stage were treated by the drugs. Two drugs were tried, viz. achromycin in oil locally, and sulphamezathine orally. Achromycin in oil was used as local drops every two hours during the day only. Sulphamezathine was given orally. The dose was 4 tablets start, and 2 tablets 6 hourly, the total dose was 25 g.

Thirty cases have been treated by achromycin in oil and the rest treated by sulphaemzathine.

19. Investigations into the factors affecting the susceptibility of diabetes to infection under Dr. T.P. Bharadwaj at the Topiwala National Medical College, Bombay.

The following studies were undertaken :-

1. Blood corticoid levels of diabetics have been estimated. These reveal significant difference from normal.

2. Control normal rabbits and rabbits rendered diabetic by alloxan, were given intraperitoneal injections of T.A.B. suspension. The rate of rise of antibody titre was noted by agglutination technique for over 2

months. Another group of rabbits was divided into two batches — a control normal and diabetic group. To both of them (the animals selected were of approximately equal weights) large quantities of antibodies against T.A.B. were given. The initial titres were noted and the rate of their dissimilation was followed by twice weekly agglutination reaction. This has been followed, up to the present for about 2 months. In neither instance have significant differences been noted. The studies are being persued further.

3. Titres of T.A.B. agglutinating antibodies have been studied in 50 normal individuals without any history of clinical typhoid in recent past (three years). Similar studies have been started in diabetes.

4. Studies on properdin, complement levels and certain metabolic aspects of leukocytes are in progress in normal and diabetic animals and men.

20. Inquiry on biochemical studies on tumor under Dr. S.C. Roy in the Department of Applied Chemistry, Calcutta University, Calcutta

During the period under review the work has been continued with human malignant and other tissues and directed mainly towards the following lines :—

(1) Isotopic studies. (2) study of the tissue with respect to (a) free and protein amino acids, (b) glycolytic phosphate esters, (c) α-keto acids, (d) nucleic acids and PNA-P/DNA-P ratio, (e) dehydrogenase systems in the mitochondria, and (*f*) peptides of the tissue after the partial hydrolysis.

(1) Isotope studies.—*In vitro* utilization of C^{14}-1-acetate by malignant and analogous normal or fibroid tissue has been followed up in different fractions obtained chemically and separated chromatographically, wherever necessary into different constituents (a) respiratory CO_2, (b) volatile compounds, (c) carbohydrates, including Kreb's cycle intermediates, (d) lipids and (e) free and protein amino acids. Preliminary scanning has been done to detect the compound having incorporation of C^{14}, and for more accurate determination recourse has been taken to radioautography.

Comparatively heavier incorporation of C^{14} has taken place in the following amino acids in malignant tissue. Glutamic acid, serine, glycine, aspartic acid, alanine, arginine and histidine, lysine, threonine, leucine, proline, methionine sulphoxide, tyrosine. From the values of the radioactive counts it appears very likely that the incorporation is rather specific and not a general one. Radioautographic study which is under way will give more specific indications. Lipid fraction from the malignant tissue has also been very heavily incorporated as compared to that of the normal.

(2) (*a*) *Free and protein amino acids.*—Sixteen more specimens of tissue-malignant, fibroid or normal have been analyzed with respect to free and total aminoacid contents. The results have been found, more or less, the same as reported last year.

(*b*) *Glycolytic phosphate esters.*—Ten different specimens of tissue (malignant, fibroid and analogous normal) have been analyzed and the results agreed to a considerable extent with those reported last year.

(*c*) *α-keto acids.*—Twelve specimens of tissue have been analyzed with respect to pyruvate, oxalacetate and α-ketoglutarate by the chromatographic method. The acids have been identified and the process for quantitative determination has been standardized and is being applied.

(*d*) *Nucleic acid and PNA-P/DNA-P ratio.*—Ten specimens of tissue have been analyzed and PNA-P/DNA-P ratio determined. Attempt has been made to isolate the DNA from human tumor tissue and separate the purine and pyrimidine bases chromatographically.

(*e*) *Dehydrogenase systems in the mitochondria.*—Mitochondrial preparation has been obtained from the tissue and glutamic acid, alanine and glycine dehydrogenases have been detected by using TTC (2,3,5,-triphenyl tetrazolium chloride) as the electron acceptor.

(*f*) *Peptides from the partially hydrolyzed tissue.*—Proteins isolated from the tissue were subjected to partial hydrolysis, high speed centrifugation and chromatography of the different fractions so obtained. Several peptides have been located and will be studied in more details with the help of column chromatography. (Moore and Stein, *Jour. Biol. Chem.*, *211*, *p. 893*, *1954*).

21. Inquiry on synthetic media of tissue culture and the measurement of proliferation of the cells under Dr. C.V. Ramakrishnan, Faculty of Medicine, Baroda University, Baroda.

The conditions have been standardized to grow chick embryo tissues directly on glass in roller tubes using different media, such as HES, M_{150}, Eagle's medium, etc.

Several trials of experiments carried out so far show that the heart fibroblast explant can be grown in roller tubes in a purely synthetic medium.

Micro-methods have been standardized to estimate the sugar uptake, protein, total number of cells and DNA of the explant during the proliferation of the explant and a correlation has been obtained between these and the proliferation. Once the proliferation stops, the sugar uptake also stops simultaneously.

Preliminary experiments carried out show that if glutamine is omitted from the medium, the tissue proliferation is better. The evidence has been obtained to show that glutamine, when added, gets converted to glutamic acid and excess glutamic acid may come in the way of proliferation.

22. Clinical study of neuropathies under Dr. P.N. Chuttani at the Medical College, Patiala.

A total of 178 cases of various types of neuropathies have been studied, at first at Amritsar and later at Patiala. 171 have been analyzed in detail, the remaining 7 not having stayed long enough for a thorough study. It was found that the main aetiological or clinical groups met with were diabetic neuropathies in 34 patients, arsenical neuropathies in 19, Landry-Guillain-Barre syndrome in 18, subacute combined degeneration in 17, metalgia paraesthetica in 10, shoulder girdle syndrome in 7, nutritional neuropathy in 10, and the ubiquitous 'idiopathic' variety in 16. A large number of other varieties were also seen, such as those due to diphtheria (4), leprosy (3), typhoid (3), serum or post-vaccinal (3), alcohol (2), compression (2), pneumonia (1), brucellosis (1), bronchogenic carcinoma (1), cancer penis (1), acute idiopathic porphyria (1), hyperemesis gravidarum (1), carbon-monoxide (1), and amyloidosis (2). Cranial polyneuritis (2), neuropathy associated with Pink disease (1), and polyarteritis nodosa (1), were also met with and a single example of chronic progressive neuropathy was seen.

Apart from routine laboratory investigations nerve biopsies from digital nerves of the involved limbs were available for histopathological examination conducted after staining of paraffin sections with H & E, azocarmine, osmic acid or myelin stain and of frozen section with silver stain. Frozen sections were also studied under polarized light. Changes, such as increased cellularity, thickening of perineurium and or endoneurium increase of fibrous tissue, fragmentation, granularity, ballooning and absence of myeline and swelling, fragmentation, diminution or absence of axons were seen in various combinations. No specific histopathological picture emerged for any aetiological variety and there was no obvious correlation between the severity of histological change and the clinical severity of the neuropathy. However, pathological changes were the rule when neuropathy was well developed. A few serial biopsies from the same patients were also studied.

23. Inquiry entitled 'Relative value of rest and movement in the treatment of intra-articular fractures : an experimental study' under Dr. B. Mukopadhya at the P.W. Medical College, Patna

1. *Object of the Work.*—To study by animal experiments the relative values of the treatment by rest and treatment by early movement in cases of operatively induced intra-articular fractures and thus determine from such experimental studies the correct method of treatment of such fractures in human beings which occur as a result of accident. The treatment of such fractures in human beings is still a controversial issue and the evidence on which the different methods of treatment are based is entirely clinical.

2. *Conditions of the work done so far.*—So far operations have been performed in three different joints of goats, the joints being the wrist, the knee and the ankle. Only these joints were selected as it is not possible to immobilize the other joints in goat. The plan of work

has been slightly altered since the submission of the report last year. The cases have been grouped into three types of fractures for each joint, as follows :-

(1) Linear fracture without displacement.

(2) Linear fracture with displacement of various degrees.

(3) Comminuted fractures with multiple fragments and marked displacements.

The experiments have been so designed that for each joint immobilized after operation the same joint in a normal animal has been immobilized for the same period of time and in exactly the same fashion. For the present study the period of immobilization has been kept fixed at 4 weeks.

It will be seen from the study so far made that in intra-articular fractures of moderate severity without comminution, immobilization of the joint very definitely prolongs the period of disability. Encouragement of early physiological activity in the traumatized joint not only reduces the period of disability considerably but also produces more perfect end results, the movement in the joint returning to full normal range. With immobilization of the joint, a certain percentage of cases develop varying degrees of stiffness in the joint permanently. This difference in the results occur with only 4 weeks immobilization—a period too short to produce complete union in the fracture.

Where an intra-articular fracture is severely comminuted causing gross degrees of mal-alignment of the articular surfaces, perfect restoration of function is not possible, whatever method of treatment is used.

24. Inquiry on tropical pulmonary eosinophilia under Dr. M.V. Chari, at the Kasturba Medical College, Mangalore.

1. A report is given on the age, symptoms and laboratory findings of 33 males and 4 females treated under this inquiry from the 1st December, 1956 to 30th September, 1957 at the Government Wenlock Hospital, Mangalore.

2. Particular reference is made to the low grade temperature with raised eosinophil count.

3. It was observed that the paroxysmal attacks bore no relation to peripheral eosinophils.

4. Bone-marrow study was undertaken in 13 cases and was followed up in 6 cases. The results of this study are not conclusive.

5. The radiological findings of 41 cases with changes noted in the appearance of the skiagrams of 20 cases followed up are given.

6. Treatment was given with (*a*) Hetrazan (*b*) Banocide, (*c*) Entacyl, (*d*) Carbarsone, (*e*) Hetrazan and carbarsone, (*f*) Antimony, (*g*) T.A.B. vaccine, and (*h*) Strontium bromide. With the exception of the last a few patients responded to tratment both clinically and hæmatologically with each of the above lines of treatment.

25. Inquiry to investigate the mechanism of antibacterial action of specific antiserum under Dr. J. C. Ray at the Indian Institute for Biochemistry and Experimental Medicine, Calcutta

Since the last report work on one of the aspects, namely, the effect of anti-serum on the lactose utilization by nucleotides stimulating adaptation in anti-serum treated cells is nearing completion.

(a) *Effect of antiserum on lactose utilization.*—It has been observed that the anti-serum treated non-adapted cells, after being pre-adapted to lactose, would utilize lactose to a much lesser degree as compared to those exposed to normal serum or the lactose adapted cells.

2-4-Dinitrophenol in a concentration of 10^{-3}M was found to inhibit the uti[illegible]

contro[illegible]

DNP [illegible]

unnoticed non-specific reduction of methylene blue through to a very small extent. This prompted the investigators to search for another redox dye which will be more specific in reduction. At the present moment, 2-3-5-triphenyl tetrazolium chloride is being used as a substitute and preliminary experiments have shown it to be a highly efficient tool for bacterial adaptation studies as required in this investigation.

(b) *The effect of adenosine triphosphate (ATP) and other nucleotides in stimulating adaptation in anti-serum treated cells:* The progress of this part of the work was retarded considerably due to non-availability of the nucleotides in pure forms. It has recently been taken up and is being studied using the tetrazolium technique.

26. Studies on metabolism of the catechol amines in bronchial asthma and tropical eosinophilia under Dr. H.S. Chakravarti at the Indian Institute for Biochemistry & Experimental Medicine, Calcutta.

Scanty reports are available on the pattern of catechol amine excretion in various disease conditions, specially where physiological discharge of adrenal medullary hormones or their defective utilization in the body may be suspected, such as in bronchial asthma and in tropical eosinophilia. Aetiology of the symptom complex of tropical eosinophilia, still remains obscure. High count of blood eosinophils and occasional improvement with ACTH and corticosteroids, rather suggest a possibility of some dysfunction of the adrenal glands. It is well known that both the cortical and medullary hormones have eosinopenic effect, the later working through the pituitary-adrenal axis.

Considering all these facts, it was decided to investigate both the cortical and medullary function of the adrenals in cases of tropical eosinophilia and bronchial asthma. Investigations were made to find out the pattern of catechol amine excretion and 17-ketosteroid excretion in 24-hour urine, before and after treatment of tropical eosinophilia and bronchial asthma. Attempts were also made to reproduce tropical

eosinophilia in guinea-pig which, however, failed. Plasma protein pattern by paper electrophoresis were also studied in these cases.

CATECHOL AMINES EXCRETION IN 24-HOURS URINE.

In tropical eosinophilia.—Patients with characteristic symptoms of tropical eosinophilia were investigated clinically, radiologically and hæmatologically in the department for confirmation of the diagnosis. In a typical case, massive leucocytosis with eosinophils not less than 2,000/cu. mm. of blood were considered for selection. All the patients included in this work came to us before any specific treatment with arsenicals. They were subsequently treated as ambulatory patients with organic arsenical preparation (acetylarsan).

For normal control, 26 healthy individuals from laboratory workers were selected. They were ordinarily considered as free from any disease and did not show any high initial eosinophil count.

Urine collection.—Twenty-four hour urine collections were made with 5 c.c. of 12 N HCl as preservative. When immediate extraction was not possible, samples of urine were stored at 4°C. in a refrigerator for not more than 7 days. In cases of tropical eosinophilia, estimations were done before instituting any treatment and again one week after completion of the treatment with arsenical.

Extraction.—According to a slightly modified method of Euler 200 c.c. aliquot of urine with 20 c.c. of concentrated HCl was boiled for 20 minutes in a reflux condenser and filtered. To the filtrate was added 3.75 g. of aluminium oxide and alkalinized with concentrated NaOH solution till a pH of 8.5 was attained. A magnetic stirrer was used to shake the mixture continuously during alkalinization. The mixture was taken in a Buchner funnel on two sheets of filter paper and put to mild suction and the filtrate was discarded. The adsorbed amines on the alumina in the funnel was washed with distilled water followed by elution with 0.25 N H_2SO_4. The eluate was collected and its pH was adjusted to 4 by adding 10 per cent sodium bicarbonate solution. The volume was made to 10 c.c. with distilled water and stored in sealed ampoules in frigidaire/deep-freeze for biological assay. Recovery with this method gave satisfactory result in our laboratory.

Assay of catechol amines in the eluate.—As the amount of catechol amines excreted in urine is very small and the calorimetric method of estimation lacks in sensitivity at such concentration, the biological method of assay was adopted. Response produced by given doses of a standard preparation of adrenaline solution in spinal cat was compared to that produced by a quantity of unknown solution and thus the pressure activity of the unknown solution was measured in terms of adrenaline. Total 24-hour content in urine was calculated from the results thus obtained.

Results

In 26 normal adult subjects, 22 males and 4 females, the range of pressor amines was 10.7 to 90.3 microgramme equivalent of adrenaline, the average being 34.8 microgrammes per 24 hours. Standard deviation was ±21.1.

The excretion in 24 cases of tropical eosinophilia 20 males and 4 females before treatment was between 8.4 and 70.9 microgrammes equivalent of adrenaline per 24 hours, the mean value being 33.5 ± 16.8 microgrammes. The excretion after the patients completed their treatment, was in the range of 10.2 to 90.0 microgramme equivalent of adrenaline, the mean value being 40.6 ± 22.7 microgrammes.

When these data are subjected to 't' test, it was found that the variations observed amongst the groups—Normal, cases before treatment, and after treatment, were not statistically significant as will be evident from the following Table:—

Table

Groups compared	't' values	Remarks
Normal *vs* before treatment	0.118	Not significant
Normal *vs* after treatment	0.461	
Before treatment *vs* after treatment	0.775	

Bronchial asthma.—Only 5 cases has been investigated and catechol amine excretion in 24-hours urine is as below in microgramme equivalent of adrenaline in 24 hours :—

Case No.	Before treatment	After 4 to 6 months symptomatic treatment
1	27.7	29.7
2	90.0	57.1
3	26.0	29.6
4	45.0	
5	87.4	31.5

It will be evident from above that as in tropical eosinophilia, in bronchial asthma also there is no significant variation in catecholamine excretion.

Excretion of 17-Ketosteroids in Urine

Although variations of excretion of 17-ketosteroids, which have got a multiple source of origin, cannot be considered as an adequate indication of altered function of adrenal cortex, as a first step to study the role of adrenal cortex in the pathogenesis of tropical eosinophilia, 17-ketosteroids were estimated in 19 cases and compared with normal values obtained in our laboratory in 21 cases. These 19 cases were also followed up after completion of their treatment. For convenience the male cases within the age group of 20 to 45 years only are included in

this study. Number of female cases are, so far, too small for comparison. Results are as below :—

Group	17-ketosteroids 24-hours urine (mg.)
Normal (21 cases)	7.44±2.48
Tropical eosinophilia before treatment (19 cases)	4.69±1.83
Tropical eosinophilia after treatment (19 cases)	5.97±2.53

Only moderate reduction in excretion of 17-ketosteroids has been observed. Such reduction has also been observed by others in various chronic illnesses and in hospital patients. Returning to normalcy after treatment indicates that the disturbance in 17-ketosteroids is functional in origin.

In 5 cases of bronchial asthma of which 4 were females—results were 4.0, 2.5, 1.5 and 5.9 mg. and one male 6.8 mg. 17-ketosteroids in 24-hours urine.

Paper Electrophoresis of Serum Protein

Electrophoretic pattern of serum proteins was studied in only 13 cases of tropical eosinophilia and the percentage distribution was analysed with planimeter after properly staining the paper strips. In general, it may be said that there is reduction in the albumin fraction and rise in the globulin of which gamma globulin showed marked rise. In 6 cases, which were studied after treatment, albumin fraction went up over the previous value and gamma globulin fraction showed distinct reduction.

Animal Inoculation

Oxalated plasma from 13 cases of tropical eosinophilia were injected intraperitoneally into fresh guinea-pig with low initial eosinophilic count. Total w.b.c. and differential counts were made every week and special care was taken to exclude the 'Kurloff' cells. All the guinea-pigs were followed for at least three months and in some cases even up to 6 months. There had been some initial rise in the eosinophil count following injection of plasma but, the rise was not sustained and the rise of count was also not to a marked extent. Moreover, in one guinea-pig injected with plasma from a patient of bronchial asthma, the sustained rise of eosinophil count observed was more than in any other guinea-pig in the previous group, indicating that the rise of eosinophilic count observed may be a non-specific reaction rather than due to a transmissible factor.

In order to investigate further the possibility of a transmissible factor, plasma from the guinea-pig showing marked rise of eosinophil count following plasma injection from the cases of tropical eosinophilia,

vas reinjected into other fresh guinea-pigs. Periodic blood count of hese guinea-pigs (in 3 cases) did not show any rise in eosinophilic ount.

7. Experimental study of the role of adrenal cortex in the genesis of congenital abnormalities under Dr. I.P. Agarwal at the G.R. Medical College, Gwalior.

Fertilized eggs of two strain of hens, i.e. white leg horn (W.L.H.) nd Rhode Island Red (R.I.R.) were used for the experimental study. he incidence of spontaneous congenital anamolies was 2 and 26 per ent in W.L.H. in R.I.R., respectively. The comparative suseptibility f fertilized eggs of two types of strains, one with low and the other with igh incidence of spontaneously developing congenital abnormalities in hick embryos to various teratogenic agents, differed in both the strains. he following are the preliminary observations :—

1. Th[illegible] the albumin [illegible] congenital [illegible] early bring ut the teratogenic action of cortisone acetate :—

R.I.R. hen number	Percentage of spontaneously occurring abnormalities in fertilized eggs in the Control group	Percentage of cortisone induced abnormalities in the fertilized eggs
1	23	55
2	29	All embryos died before 10th day
3	44	-do-
4	14	100
5	0	20
6	25	40

The action of cortisone acetate on the developing chick embryos aried not only with the degree of susceptibility of eggs from different ins, but also on the age of the developing chick embryos at the time the injection

2. Various abnormalities occurring in the chick embryos were nomelia club foot, beak deformities, microphthalmia, unilateral or lateral absence of eyes, dwarfism, ectopia viscera and exencephaly hese abnormalities were almost similar to those occurring spontaneously d induced with eserine sulphate and insulin.

3. 10 mg. of cortisone was injected into the hens and the fertilized eggs from these hens were collected for 1½ months and incubated as such. The following were the observations :—

PERCENTAGE OF SPONTANEOUS ABNORMALITIES

Hen number	Before injection of cortisone acetate	After injection of 10 mg. of cortisone acetate
1	23	0
3	44	0
6	25	0

From the above data, it appears that cortisone acetate, if injected into the hens, exerts an anti-teratogenic action.

4. The congential abnormalities in the chick embryos have been successfully induced with eserine sulphate and insulin.

From the preliminary observations it appears that cortisone acetate, the active hormone of the adrenal cortex, can modify morphogenesis and development of the growing chick embryos, depending upon the susceptibility of individual hens, dose of cortisone acetate, and the age of developing chick embryos at the time of injection.

28. Study of the mode of infection and growth characteristics of rhinosporidium seeberi under Dr. Indu Mohan Gupta at the Kasturba Medical College, Mangalore.

Two cases of rhinosporidial growth, both from nose, came under investigation during the period April, 1957 to September, 1957. Specimens removed at operation were collected in sterile physiological saline. One part of the growth was subjected to histopathological study. From the remaining portion, attempt was made to grow the causative organism on different artificial culture media and also tissue transplants of the growth were made aseptically into the buccal mucosa of the guinea-pigs. Attempts to grow the organism have so far not been successful.

During the course of cultural study it was interestingly observed that the wall of the sporangium of rhinosporidium seeberi is markedly resistant to prolonging desiccation. The resistant nature of the wall of the sporangium in general, may presumably be related in some way to the failure in obtaining the rhinosporidial growth on artificial culture media and in tissue transplant experiments.

29. I[illegible] for culturing [illegible] so far under [illegible] College, Jaipur.

It was thought that *Rhinosporidium seeberi* could grow either on special media or in the living tissue of the animals, such as monkeys and rabbits. For these purposes a small tissue was excised from the *Rhinosporidial* polyp in the nostril of the patient. It was then immediately, but gently, broken up in brain heart-infusion liquid medium in a sterile glass pestle and mortar.

A part of the suspension was inoculated on specialized media containing human blood and growth factors, such as yeast, cystine and Biotin. A part of the suspension was then injected into the mucous membrane of the nostril of the monkey and the rabbit. During this period four cases of *Rhinosporidiosis* attended the Hospital but I could get material from three cases only and this was injected into the nasal mucosa of the monkey and the rabbit.

All attempts to grow the organism on artificial medium containing human blood and growth factors, such as yeast, cystine and Biotin did not yield any growth of *Rhinosporidium seeberi*.

No growth or abnormality has appeared as yet in the nasal mucous membrane of either the monkey or the rabbit. They are being kept for further observation.

Results

1. All attempts to grow the organism on special media have failed.
2. No growth has yet appeared in the nasal mucosa of the monkey and the rabbits inoculated with a suspension of the excised polyps.

30. Investigation of antistreptolysin titres in children under Dr. S. L. Bhatia at the Lady Hardinge Medical College, New Delhi

Attempts at preparing *Streptolysin O* in the laboratory were not successful. The group A *Streptococcus* obtained from Colindale (National Collection of Type Cultures) was found not to be viable and the strains isolated from cases investigated in the laboratory were used and were not satisfactory.

A small amount of *Streptolysin O* was obtained from U.S.A. A certain number of sera were put up and the results were as follows:—

Normal cases of children with no history of recent sore throat.

Case No.	*Antistreptolysin titre*
N. 1	80
N. 2	60
N. 3	80
N. 4	20
N. 5	0
N. 6	100
N. 7	100

Active rheumatic cases—majority from Himachal Pradesh obtained by Dr. Devi Chand	*A.S.O. titre.*
Case No. R. 1	1,000 Units.
R. 2	100 ,,
R. 3	1,000 ,,
R. 4	2,000 ,,
R. 5	4,000 ,,
R. 6	1,000 ,,
R. 7	1,000 ,,
R. 8	1,000 ,,
R. 9	1,000 ,,
R. 10	600 ,,
R. 11	2,000 ,,
R. 12	4,000 ,,
R. 13	4,000 ,,
R. 14	4,000 ,,
R. 15	800 ,,
R. 16	2,000 ,,
R. 17	4,000 ,,
R. 18	2,000 ,,
R. 19	2,000 ,,
R. 20	800 ,,
R. 21	1,000 ,,
R. 22	100 ,,
R. 23	1,000 ,,
R. 24	1,000 ,,
R. 25	4,000 ,,
R. 26	2,000 ,,
R. 27	80 ,,
R. 28	1,000 ,,

A large number of sera have been collected and await investigation.

31. Comparative study of serological techinques in detecting antibodies following immunization with *S. Typhi 0* antigen in rabbits and sera of patients suffering from enteric fevers under Dr. A.K. Banerjee at the S.S.K. Memorial Hospital, Calcutta.

Preliminary work on the immuno-chemistry of the specific polysaccharide hapten of the 0 antigen of the smooth form of *S. typhi*

is in progress. A non-motile 0 variant of smooth form of *S. typhi* was used in the investigation. A method has been standardized for preparing an aqueous extract of the 0 antigen of *S. typhi*. The specific polysaccharide was obtained by acetone treatment of the quantitative [illegible] determined. The effect [illegible] ration of growth on [illegible]

32. Inquiry entitled 'Early diagnosis of enteric fevers' under Dr. N. P. Gupta and Dr. S. P. Gupta, at the Medical College, Lucknow.

(1) Experiments were carried out to determine the presence of soluble polysaccharide antigen of *S. typhi*.—

(*a*) In the urine of patients of typhoid fever.

(*b*) In the sera of patients of typhoid fever.

(*c*) In the sera of mice inoculated with *S. typhi murium*.

(*d*) In the urine and fæces of mice inoculated with *S. typhi murium*.

Chicken red cells were sensitized with cell free extracts of *S. typhi* and *S. typhimurium* as required for different tests. Those sensitized red cells were used as agglutinable suspensions. A standard '0' serum of known titer was put up in a standard volume in doubling dilutions. In a parallel titration a suitable volume of urine, serum or fæcal extract was added. The titre of the standard serum in the presence of hypothetical soluble antigen was noted and recorded.

[illegible] tests were carried out with the [illegible] sera and fæcal extracts of [illegible] experimentally), to detect the presence of soluble antigen.

Urine of 8 patients of typhoid fever with a positive blood culture was investigated, and the results showed that there was some indication of presence of soluble antigen in 5 out of 8 cases.

Hæmagglutination inhibition tests were carried out with sera of 16 patients in which blood culture was positive. The results indicated that only in 3 cases there is some indication of presence of soluble antigen.

(2) The technique of production of experimental mouse typhoid has been standardized, and viable counts carried out to determine the phases of bacteræmia. Mice were kept in metabolic cages so that their urine and fæces could be collected easily. Preliminary tests have been carried out to determine, if urine, fæcal extract and serum from mice with maximal bacteræmia would cause inhibition of hæmagglutination test with standard serum prepared against the antigens of *S. typhi murium*.

33. Inquiry into the problem of chronic splenomegaly and its relation to hepatic pathology under Dr. B. K. Aikat, and Dr. A. K. Basu at the Institute of Post-Graduate Medical Education and Research, Calcutta.

The work was carried out according to the approved plan of study. (1) *Clinico-pathological study.*—A total of 31 cases of chronic splenomegaly have so far been investigated in detail. Twenty-nine of these cases have been operated on and are being followed up.

RESULTS.

The data obtained are summarized as follows:—

	Number of cases
(1) *Pre-operative* :	
(a) *Clinical* :	
Significant history of fever	9
Haemorrhage-hæmatemesis and melaena ...	16
Jaundice	1
Pallor	31
(b) *Heamatological* :	
Anæmia	31
Normocytic normochromic	19
Microcytic hypochromic	12
Leucopenia (below 3,000/cmm.) ...	20
Thrombocytopenia (below 100,000/cmm.) ...	7
(c) *Bone-marrow study* :	
Hyper-cellular marrow	29
Normoblastic	29
Hypo-cellular normoblastic	2

(d) *Liver function studies.*—A battery of liver function tests were performed which included total proteins, serum albumin and globulin by micro kjeldahl method, electro-phoretic study of serum protein by 'moving boundary technique' thymol, zinc sulphate turbidity tests, cephalin cholesterol flocculation tests, total serum bilirubin and alkaline phosphatase.

On the above basis liver function was considered :—

	No. of cases
Normal	8
Moderately damaged	9
Severally damaged	14
(e) *Radiology :*	
(a) Ba swallow	14
Oesophageal varix	

	No. of cases
(b) *Splenoportal venography :*	
(i) Extra hepatic obstruction ...	9
(ii) Dilated tortuous splenic vein without collaterals	6
(iii) Dilated tortuous splenic vein with collaterals	16
(2) *Operative study.*	
(a) Portal venous pressure :	
(i) Below 200 mm saline	2
(ii) 200-300 mm saline	10
(iii) Above 300 mm saline	15
(b) *Appearance of liver :*	
Coarsely nodular	14
Diffuse granularity	4
Wide scarring	2
Atrophy of left lobe	2
Fatty liver	2
Enlarged without granularity	2
Normal appearance	5
(c) Presence of thrill in splenic artery ...	6
Operative procedure :	
Splenectomy alone	16
Splenectomy with splenorenalshunt ...	9
Porta-caval shunt	1
Ligation of hepatic artery	3
Final aetiological assessment of cases.	
(a) Cirrhosis of liver with portal hypertension and fibro-congestive splenomegaly	20
(b) Extrahepatic hepatic obstruction with portal hypertension and fibrocongestive splenomegaly	9
(i) Portal venous thrombus .	1
(ii) Cavernomatous transformation of portal veins	3
(iii) Chronic pancreatitis	1
(iv) Pancreatic tumour	3
(v) Hypertrophic caudate lobe pressing on portal vein	1

(c) Hyperplasia of the Reticulo endothelial cells of the spleen.

			No. of cases.
(i) Kala-azar	...	...	1
(ii) Unknown...? Malaria	...	...	1

Histology of the liver :

Post necrotic cirrhosis	...	...	14
Diffuse hepatic fibrosis	...	...	4
Post necrotic scarring	...	...	2
Protein malnutrition	...	...	2
Fatty liver	...	...	5
R.E. hyperplasia	...	...	3
Normal structure	...	...	8

Histology of the spleen :

Fibro congestive-splenomegaly		...	24
Primary R.E. endothelial-cells hyperplasia		...	3 including 1 K.A.
R.E. hyperplasia in association with fibro-congestive splenomegaly	...	...	24
Myeloid Metaplasia	...	...	11
Infarcts	...	...	7

Follow up study.—This consists of periodical and regular clinical, hæmatological, radiological examination of the patient combined with evaluation of the structural and functional status of the liver.

Experimental work in animals.

(1) *Monkeys :* No. of cases.

Supra and infra hepatic obstruction—
Mechanical with cellophanetape :

Supra hepatic obstruction	...	...	6
Infra hepatic obstruction	...	...	4

(2) *Infra hepatic obstruction :*

Production of cirrhosis in Albino rats by injection of 0.1 ml. of carbon tetrachloride per 100 gm. of body wt. once a week	...	36

(3) *Splenic hyperplasia :*—

Biweekly injection of 0.2 cc. per 100 g. of wt. of 2.5 per cent methyl cellulose of viscosity grade of 400 centipoises.

Normal diet and methyl cellulose	...	...	18
Low protein diet (2 per cent protein) and Methyl cellulose	...	...	18
Low protein diet alone	...	...	18

The data from the experimental work cannot be analysed at the present stage.

There is good deal of suggestive evidence that there is marked increase in the splenic blood volume and outflow in most of the cases with presence of a distinct thrill in the splenic artery in some cases

34 Inquiry into electrophoretic study of immunised rabbits with particular reference to immunological tolerance under Dr D Barua at the S S K. Memorial Hospital, Calcutta

The term immunological tolerance refers to a specific weakening or suppression of re-activity caused by the exposure of animals to antigenic stimuli before the maturation of the faculty of immunological response. Rabbits have been selected as the experimental animal and *S. typhi* as the antigen to see whether these animals develop immunological tolerance to this bacterial antigen when challenged in their foetal or early life. The experiments so far carried out are described under five headings :

(a) Four adult rabbits of about four months of age were inoculated [illegible] nd 200[illegible] of heat killed *S. typhi* [illegible] were varying from 1:5120 to [illegible] ectrophoretic study was done by 'boundary zone' method in an Antweiler apparatus and showed high peaks of gamma-globulin. Absorbed sera with homologous antigen showed fall of the gamma-globulin peak.

(b) Electrophoretic pattern of sera of five adult and 10 young un-immunized rabbits was studied by the same method. The electrophoretic globulin pattern of one and two days old rabbits was found to be different from that of adult rabbits.

(c) Two pregnant rabbits were immunised with *S typhi* antigen by the same method as was employed in the experiment A commencing from the 15th day of gestation. The sera of their 7 baby-rabbits (3 from one litter and 4 from another) were found to contain antibodies against *S typhi* and the titre was found to be between 160 and 1:320. When they were 3 months old the titre of their sera fell to 1:20 to 1:80. Out of these 7 *litters 2 died Remaining 5 litters were available for subsequent* immunization when they were of 4 months age. The schedule of immunization was same as in their mothers. Two of them showed rise of titre to 1:160 and in 3 others the titre went upto 1:320

(d) Two embryo[illegible] uteri on the 2[illegible]th day of gestation [illegible] they died after birth. The other one [illegible] tested 6 weeks after birth. This animal was immunized by the 3 injections [illegible] against *S. typhi* and the titre did not go above 1:320.

(e) Out of 4 litters (13 rabbits in all), 9 were injected [illegible] with 25[illegible] organism within 1 to 16 hours of their birth and one from each of the 4 litters were kept as control without any [illegible]

dose. Four of the 9 test animals died within 3 or 4 days. Remaining 5 test animals and 4 control animals did not show any antibody when their sera were tested with *S. typhi* antigen 6 weeks after birth. All the 9 animals (5 test animals and 4 control animals) were then immunized with the 3 injection schedule. All control animals (four) developed antibodies, the titre varing from 1:2560 to 1:5120. In the test animals (five), the titre varied from 1:160 to 1:320. It is not possible to try to draw any conclusion about the state of immunological tolerance against bacterial antigens in rabbits from the experiments that have so far been done. Attempts are being made to increase the number of animals in each experiment and to evolve new experiment to prove or disprove this.

35. Study of kwashiorkor with special reference to histopathological and histochemical changes in the cutaneous lesions under Dr. C. Mohan Rangam at the M.G.M. Medical College, Indore.

The material studied so far consists of skin biopsies from 8 children with typical cutaneous lesions of kwashiorkor and 4 normal healthy skins. The criteria for selection of cases were the presence of œdema, retarded or arrested physical growth, mental apathy, enlargement of liver, hypoalbuminæmia, and typical cutaneous lesions, i.e. crazy-pavement dermatosis, enamel paint dermatosis, parchment dermatosis and crackled skin appearence. In all but two cases, biopsies were taken from the skin of the abdominal wall. In the other two cases, biopsies were taken from the lateral aspect of the leg.

The sections from the formal saline processed tissue were stained with hæmatoxylin and eosin for routine study. The collagen, elastic tissue and melanin were studied by Van Gieson, Verhoff and Fontana stains, respectively. The sections from alcohol-fixed tissue were stained with congo red hæmatoxylin for keratohyaline and eleidin.

It has been observed by previous workers that whilst pellagrous lesions occur in regions exposed to sunlight, kwashiorkor lesions seldom do so. We are unable to confirm this observation since in our series the lesions have been observed over the dorsum of hand and forearm and extensor surfaces of the legs, distribution regarded as typical of pellagra.

Histologically, changes have been observed in the epidermis and dermis. In the epidermis, the most striking feature was atrophy of which the following patterns could be recognized.

Epidermis.—

(*a*) Marked and uniform atrophy with no hyperkeratosis or parakeratosis.

(*b*) Irregular epithelial atrophy alternating with normal stretches causing a marked corrugation of surface.

(*c*) Epithelial atrophy with co-incidental hyperkeratosis and parakeratosis.

In areas showing marked atrophy, the stratum malphigi and stratum basalis are so greatly decreased in thickness that the entire thickness of the epidermis seems to be constituted by strata granulosa and lucida. In the atrophic areas the stratum granulosum is depleted of keratohyaline granules.

The disturbances in the basal layer seen maximally in the atrophic areas but elsewhere also consist in an apparent displacement of these cells and loss of cohesion between them. The melanin pigment is absent in the basal layer and is dispersed in the rate malphigi and also in dermis. In the latter case the pigment is found lying either loose or held by histiocytes.

Dermis.—The fine wavy collagen of the papillary layer shows an [illegible] of the fibrils and fragmentation. The [illegible] the sub-papillary layer also show [illegible] show fragmentation and clumping. There is generalized infiltration of the dermis with histiocytes best seen in the papillary layer.

Even at this stage the cutaneous lesions observed in kwashiorkor seem to be attributable to multiple deficiencies. The hypoproteinemia observed by us would explain the oedema in the dermis. Perhaps, the increased permeability of the dermal capillaries due to deficiency of vitamin C reported by previous workers would aid and aggravate the development of oedema. The latter appears to be responsible for fragmentation of collagen and elastic tissue.

The epithelial atrophy is the result of dermal oedema and the consequent compression of the dermal capillaries feeding the epidermis. The hyperkeratosis and parakeratosis is explainable on the basis of vitamin A deficiency and perhaps vitamin B complex deficiency. Keratohyalin granules, being protein in nature, are depleted in kwashiorkor, a disease of protein deficiency.

Further, it seems possible to correlate the clinical appearence of any particular type of lesion with the specific histopathological changes, e.g. in parchment dermatosis there is uniform and marked epithelial atrophy, in crazy-pavement dermatosis or enamel paint dermatosis, there is combination of hyperkeratosis and parakeratosis with atrophy, and in crackled skin appearence there is marked corrugation of the epidermis. The simultaneous incidence of atrophy and hyperplasia has not been reported in pellagra.

36. Studies on the role of inositol in hyperlipemic conditions under Dr. V. Srinivasan at the Madurai Medical College, Madurai

The influence of inositol on lipid metabolism was found to be associated more with the cholesterol metabolism than with the other lipid fractions. Hexa-chlorocyclohexane (Gammexane), the anti-metabolite for inositol was found to inhibit the cholesterol esterase activity of liver and pancreatic extracts. This inhibition was found to be reversed almost completely by inositol.

37. Study on the effects of selected respiratory stimulants in states of pulmonary insufficiency associated with hypercapnia and hypoxia under Dr. N.R. Konar at the Nilratan Sircar Medical College, Calcutta.

Ten cases of pulmonary insufficiency were taken. In addition to a detailed clinical survey, respiratory function tests, including spirometry and arterial blood gas analysis, were done in them before and after injection of 0.24 g. of aminophylline slowly by intravenous route.

Oximeter was calibrated against van Slyke manometric apparatus.

Clinically, all the cases showed signs of emphysema and after the injection of aminophylline, patients became more restful and dyspnoea was relieved to some extent. Respiratory rate was decreased in 7 cases and increased in 3 cases, but the depth of respiration was increased in all the cases. Pulse rate slightly decreased in all the cases. Slight cyanosis was present in 2 cases before injection and it disappeared after injection. Rhonchi was much less after injection.

Regarding respiratory function tests, injection of aminophylline stimulated respiration; particularly the amplitude of respiration was increased, and in all the cases there was a net increase of pulmonary ventilation. The vital capacity and maximum breathing capacity were also increased. The percentage of total vital capacity in 1st and 2nd seconds was increased in all the cases investigated. Ventilatory equivalent of oxygen, however, increased in all cases.

Oxygen content and percentage saturation of arterial blood were increased moderately. The carbon dioxide level of arterial blood showed a change in the reverse direction (In one case, injection of aminophylline was followed by 31 per cent increase in pulmonary ventilation, 37 per cent increase in oxygen consumption per minute and about 70 per cent increase in oxygen content of arterial blood. The calculated oxygen tension of arterial blood increased from 66 to 103 mm. of Hg.; there was, however, no significant alteration in the carbon dioxide figures in this case).

38. Inquiry entitled, 'Pollination Calender for Greater Delhi' at the Vallabhbhai Patel Chest Institute, University of Delhi, Delhi.

A provisional list of the allergenic plants of Delhi along with the chronology of their pollination season has been compiled. An herbarium and a reference collection of permanent pollen slides of allergenic plants which flowered during the past four months (since June 1957) have been prepared. The incidence of atmospheric pollens at three stations selected in different parts of the city has been recorded. Collection of large amount of pollens from suspected allergenic plants for the extraction of antigens is being carried on.

39. Inquiry on the relative values of colpomicroscopy and vaginal cytology in the detection of early carcinoma of cervix uteri under Dr. Chinmoy Ghose at the Calcutta National Medical Institute, Calcutta.

The detection of early carcinoma of the cervix uteri is of paramount importance to achieve maximum salvage of patients with modern armamentorium at our disposal. The conception of what constitutes the early stage has changed radically during recent years. Carcinoma-in-situ, intraepithelial or pre-invasive carcinoma before the basement membrane barrier is broken has been widely accepted. Unfortunately cervices with pre-invasive carcinoma seldom arouses any suspicion on clinical examination. Cervices that present detectable clinical lesion, diagnosis is usually established on tissue biopsy.

Cyto-diagnosis.—Cancer cells being exfoliative in nature, detection of these cells in the vaginal discharge has been universally employed because of its simplicity and wide scope of screening the total population of women to detect the incipient carcinoma. There are obvious fallacies with a cyto-negative or cyto-positive diagnosis.

Colpo-microscopy.—Hinselmann's (1924) original devise of Colposcope gives a magnified view (10-40 times) of the cervix and has been found to be useful in selecting sites for biopsy A great improvement over this magnification has been incorporated in the recent invention of *Colpo-microscope (Antoine and Grunberger) which gives a magnifi-* [illegible] great scope [illegible] f the portio [illegible] ie gynæcolo- [illegible] it is set up [illegible] of the [illegible] vantages [illegible] repeated as often as is necessary, is harmless and causes no pain, (3) the superficial epithelial layers or modified areas at the surface and slightly *deeper seated cell layers upto a de* [illegible] could be seen just one would [illegible] ordinary microscope. There ar [illegible]

The present study is aimed to find a co-relation between colpomicroscopy and vaginal cyto-diagnosis supported by tissue biopsy whenever indicated in the detection of incipient or early carcinomatous lesion of cervix.

Unselected patients of 25 years of age and over attending the out patient's department are subjected for study. Cytological smears for papanicolaou stain are made from (a) vaginal discharge collected from posterior fornix, (b) surface scraping by Ayre's spatula from Squamo-columnar junction. A hanging drop preparation is made to exclude Trichomonas infection. History, other gynæcological findings with special note of the clinical condition of the cervix are recorded Colpo-microscopy is done on the same day or at a subsequent date. When indicated cervical biopsy is taken from the areas selected on naked eye

or Colpo-microscopic findings. 8 to 36 histological sections are prepared for study. Cytological and Colpo-microscopic examination are repeated when necessary.

During a period of three months total number of patients examined were 217, of these, only cytology was done in 58 cases, cytology and Colpo-microscopy in 156 cases and cytology, Colpo-microscopy and biopsy in 24 cases. The cases may be grouped as follows :—

1. Cervix clinically healthy — 130 cases.

(a) Colpo-microscopy (C.M).
Cytology } Negative in 71 cases.

(b) Only Cytology done — Nagative in 58 cases.
(Patients failed to report for C.M. examination).

(c) C.M.—Negative
Cytology—Positive } In 1 case.

Smear showed endometrial type of malignant cells in a post-menopausal woman with healthy cervix, with normal sized uterus and unilateral adnexal mass. The presumptive diagnosis of adnexal malignancy was confirmed postoperatively as primary tubal carcinoma.

(d) C.M.
Cytology. } Both positive—no case encountered.

2. Cervix clinically unhealthy but did not appear malignant — 82 cases.

(a) C. M.
Cytology. } Both negative in 79 cases.

(b) C. M.
Cytology. } Both suspicious in 3 cases.

Cervical biopsy-negative in 2 cases by serial sections. In one case, biopsy showed moderate basal cell activity in one area. Examination of whole cervix by serial sections after total Hysterectomy did not reveal any activity in other areas. The cases are under follow up.

3. Advanced clinical carcinoma of cervix in 3 cases.
C.M. could not be done due to hæmorrhage.
Cytology—Positive in 2 cases.
Biopsy—Invasive carcinoma in all 3 cases.

4. Vulval Carcinoma—1 case.
C.M., Surface scraping smear and Biopsy were all positive.

5. Vaginal vault recurrence—1 case.
C.M.—Positive.
Cytology—Negative.
Biopsy—Positive.

The patient was followed up post-operatively after radical abdon nal hysterectomy for carcinoma cervix with pregnancy. Cyto-diagno was twice negative but colpomicroscopy revealed typical malignant ce at the shallow vaginal vault, confirmed by subsequent biopsy. The stu is under progress no conclusion can be drawn at this stage.

40. Studies to investigate the role of mucopoly saccharides in t formation of kidney stones under Dr. K N. Udupa at t Himachal Pradesh Hospital, Simla.

For this research scheme, the blood, urine and kidney biops of the normal people and those suffering from kidney stones we the mucopo ethods of t

1. *Blood* —Ten healthy members of the staff of the Himach Pradesh Hospital were selected as the control subjects for this stu The serum mucopolysaccharides as determined by the glucosami content varied from 62.5 mg. to 92.5 mg the average being 72 5 n per 100 cc. In the same persons the serum calcium and phosph rous contents were also estimated to correlate the same with that mucopolysaccharides. The serum calcium varied from 8 0 mg. 14.0 mg. the average being 11 0 mg. per 100 cc. The phosphoro content varied from 3 0 to 5.0 mgm. the average being 3.75 mg. p 100 cc.

Ten patients suffering from various types of kidney stones w also studied for the above constituents and found that serum mucopo saccharide contents of the patients varied from 58 75 to 82.5 mg. t average being 72.5 mg. per 100 cc. The serum calcium of these patie varied f 1.0 mg. per 100 cc. T serum the average being 4 0 n per 100 is at once clear that th is no significant difference between the normal and those patients w renal lithiasis so far as the blood mucopolysaccharides, calcium a phosphorous are concerned.

2. *Urine.*—All the studies in the urine were carried out in t 24 hour urine specimen. These were analysed for glucosamine conte as per the same methods with certain modifications as was done for t serum. The urine calcium and urine phosphorus excretion rates w also determined and calculated for the 24 hour period. The results this study indicate that in normal persons the mucopolysaccharid calcium and phosphorous excretion rates were 154.2 mg./24 hrs., 650 m 24 hrs and 1200 0 mg./24 hrs But in those cases with kidney stones, t findings were 72 mg. and 6024 mg. and 316 mg. per 24 hours respective From these findings one can conclude that patients with renal calc excrete lesser quantities of mucopolysaccharides and phosphorous a large amounts of calcium. This has been a consistent finding in all c cases so far studied. However, further studies in more cases will h to be done to arrive at a definite conclusion.

Histochemical studies of the kidney biopsy.—Kidney biopsies were taken from normal persons and from those suffering from kidnev stones. Normal kidney biopsies were made when we were operating in the neighbourhood of the kidney and a few fresh autopsy specimens were also taken for these studies. The biopsies of the kidneys with stones were invariably made during operation. All the specimens were studies histologically and histochemically. From preliminary observations it appeared that in the kidneys with stones there is derangement of mucopolysaccharides both quantitatively and qualitatively in distribution especially in and around the urinary tubules.

From these fidings it seemed that there is some retention of mucopolysaccharides in the kidneys which might be responsible for the initiation of nidus formation for the stones in the kidney. Their future growth and development into typical stones might take place in the calyces where the decreased mucopolysaccharides and increased calcium content of the urine might further contribute to their development.

41. Induction of gall stones in monkeys under Dr. B.N. Balakrishna Rao at the G.R. Medical College, Gwalior.

The problem of gall stones is the problem of calculus formation, in general, and cannot yet be said to have been solved. In this Laboratory, urinary stones have been successfully produced by introduction of F.B. nucleus in the urinary bladder of dogs (T.B. Singh *et al*, 1954). On a parallel study, this work was undertaken to elucidate the role of obstruction, infection and implantation of nuclei in the production of gall stones in monkeys.

Three types of nuclei (zinc, magnesium and pieces of human gall stones) have been used as intracholecystic implants. Serial studies of blood cholesterol, roentgenograms, pH of bile and histological examination of gall bladder wall are being carried out. The effect of cystic duct obstruction after nucleus implantation is also being observed.

Till the time of this report, stones have not formed. In most cases monkey bile was found slightly on the acid side and this factor might be responsible for the prevention of deposition of crystalloids (e.g. calcium carbonate).

With the idea to possess an all-time easy access of gall bladder bile for serial examination and wilful manipulation, a polythelene tube from the inside of the gall bladder to the outside on the surface of the abdominal skin is under trial in dogs. It would be worthwhile to observe the effect of controlled changes in bile pH.

42. Effect of tissue therapy in the prevention of blindness due to degenerations and abiotrophies of the retina and choroid under Dr. J. Bose at the R.G. Kar Medical College, Calcutta.

Altogether we examined 22 cases of pigmentary degenerations of retina, out of which 10 cases are primary and 12 cases are secondary. Of

all the cases examined by us, only one case was female who is a secondary one and rest are males. The age of the patients, examined by us, varied from 6 years to 44 years and the age of onset of the night blindness, (as told by the patient, or its attendant) varied from 3 years to 12 years From the genetical study, we have found that in 2 cases there is dominant heredity and in one of them *Retinitis pigmentosa* was present in the same generation. The visual acuity was examined in the modified Landolt's ring charts.

All the cases were examined clinically and investigated properly and from the investigations and clinical studies we divided the cases into primary and secondary. Out of 12 secondary cases, only one was a female. Age variation was between 13 and 46 years, while the age of onset, as obtained from the history, was between 9 and 44 years. While investigating the ætiology, it was found that 5 cases showed positive Mantoux test over 1 in 10,000 dilutions (tubercular).

Only 3 cases showed a positive Wassermann and Kahn's precipitation test (syphilitic), while in 4 cases, ætiology could not be determined. However, on clinical basis, these undetermined groups could not be included into the primary series as these patients showed a normal dark-adaptation and had no night blindness They had, on the contrary, certain amount of day blindness The perimetric observations were done in all the cases and the records of the observations were noted. The dark adaptation test was done in most cases (both primary and secondary) by means of Della Casa adaptometer

Preparation of aqueous extract of placenta.—The placenta (Caesarean or normal delivery) was kept in the refrigerator for 8 to 10 days in the same temperature, mentioned below. The refrigerated placenta was washed thoroughly with distilled water to
Then 25 g. weight of placenta was cut fr
cotyledons and then minced very thoro
was finally triturated in a mortar. 250 c.c. of sterile normal saline was then added and then kept at room temperature for one hour with occasional stirring. After that the solution was heated in a water bath (temp. 60° C.) for one hour. The solution was then passed through cotton and paper filters. The filtrate was again filtered with paper filters several times but as the sediments were still left, the filtrate was finally passed through glass-wool filter. The filtrate was then boiled in a water bath (temp. 60° C.) for 15 minutes and then sealed in glass ampoules. The sealed ampoules were then autoclaved for 45 minutes (temp. 118°C. and 15 lb weight) The autoclaved ampoules containing the solution were then kept in a refrigerator again (temp. 2°C to 4°C.). The protein-content of the placenta was then examined and finally ærobic and anærobic culture examination was done before injecting to patients. Before injecting the solution to human beings it was injected to a guinea-pig to see any reaction. The sealed ampoules were always kept in refrigerator. Unfortunately, turbidity and reddish colour of the filtrate could not be driven out. We are still investigating about the causes of these defects specially the higher percentage of protein than Filatov's specification ('01 percent).

TECHNIQUE OF TISSUE THERAPY

(A) *Grafting.*—At first, the grafting was done in the subtenon's space (*vide* comprehensive report submitted in 1956). Next the sub-conjunctival spaces were tried but due to extreme fibrosis the technique was modified and implantation done in the subcutaneous space of the anterior abdominal wall. Later, this was changed to the proximal portion of the ventral aspect of the forearm. When the implanted tissue was bigger than the size of a pea the stitches gave way due to tension and the implanted tissue came out and as such we implanted less placental tissue and made the surgical wound bigger.

The placenta grafting was always done under local anæsthesia and under strict aseptic measures. In the subconjunctival and subtenon's route there was sometimes pain after the action of the local anæsthesia was over and the congestion used to last for more than 5/6 days but there was no infection and coming out of the tissue except once or twice. But in the subcutaneous route, there was no pain during and after operation excepting one who felt too much pain after the operation but there was no infection. There was no stitch abscess except in only one case.

We usually graft 4 times in a year at an interval of 3 months.

(B) *Injection of aqueous extract of placenta.*—The aqueous extract of placenta (so far of Albert David & Co. Ltd.) was injected by intra-muscular route daily. Forty five such injections were done at a stretch during which one implantation was done along with the injections of aqueous extract. Four such courses were done in a year. The ampoules were always kept in a refrigerator at a temp. of 2°C. to 4°C. Sometimes patients were supplied ampoules with an advice to keep them in ice flask. There are no untoward symptoms with the injections of the aqueous extract of placenta upto this day.

43. Inquiry into blood stream cooling as a method of inducing and maintaining hypothermia under Dr. A.K. Basu at the S.S.K.M. Hospital, Calcutta.

Thirty-five animal experiments have been carried out during the period from May to December 1957. The experiments have been divided into 3 categories.

1. STANDARDIZATION OF TECHNIQUE OF EXTRA-CORPOREAL VASCULAR COOLING FOR HYPOTHERMIA AND STUDY OF THIS METHOD OF COOLING AS CONTRASTED WITH THE SURFACE METHOD.

Twenty-six experiments were carried out under this category. The technique employed in per vascular cooling was as follows :—

(*a*) Cannulation of femoral artery and vein by polythene cannula.

(*b*) Circulation of the blood by means of a hand pump through a latex tube submerged in brine mixture (average temp. — 4°C).

(c) Heparinization of the animal's blood to prevent intravascular clotting.

(d) Electro-cardiographic hook up.

All these experiments were carried out with the animal under I.V. pentothal anæsthesia with endotracheal intubation.

In addition, in 2 experiments, lytic cocktail consisting of pethidine, phenergan and chlorpromazine was used.

A number of difficulties were met with during the carrying out of this experiment These included :

(a) Thrombosis of blood in the cooling system.

(b) Air embolism.

(c) Deficient cooling due to insufficient cardiac action in the absence of a pump in the system.

(d) Sudden ventricular failure due to loss of peripheral resistance.

These were gradually overcome as experience was gained in the experiments.

It was found that the per vascular method of cooling was generally slower than surface method of cooling and entailed a number of difficult technical procedures which were sometimes associated with complications.

2. Utilization of technique of hypothermia for creation and correction of intracardiac defects

Method Under [illegible] was lowered to 28°C [illegible] id inferior vena cava [illegible] was divided.

After pericardiotomy, the cardiac inflow was occluded and the right atrium opened. The interatrial septum was exposed, and a standard size defect created. The atrial wall was closed and the cardiac inflow was re-established by releasing the loops The average time of cardiac inflow arrest was 7 to 9 minutes.

Three animals survived the procedure and 6 died. No death was due to ventricular fibrillation or attributable to the direct effects of hypothermia

3. Biochemical and histological studies of different types were undertaken. Blood sugar, blood urea, blood uric acid, liver glycogen, and myocardial potassium were estimated in a number of experiments. There was a general tendency towards rise of blood sugar and fall of liver glycogen and of myocardial potassium. Blood urea and uric acid did not show any significant variation.

Histological studies were made in a number of experiments of different parenchyma, such as liver, lungs, myocardium, and kidney. No definite information can be given at present.

44. Inquiry under investigation into the pathogenesis of spinal concussion following injuries of spine under Dr. H.K. Sarkar at the S.S.K.M. Hospital, Calcutta.

The following methods were tried to produce spinal concussion in rabbits :—

1. The dura in the dorsal region was exposed by laminectomy under local anaesthesia (1 per cent novocain 7 c.c. to 10 c.c.). The cord enclosed in the dura was traumatized by the tip of an artery forceps. Six animals were used.

2. Acute forcible flexion of neck was attempted under *x*-ray screening in another 6 animals. It became apparent that it is almost impossible to dislocate or fracture a cervical vertebra.

3. This was done in 2 stages.

Stage I.—The idea of this stage is to impair the stability of the cervical spine. This was done under local anæsthesia by separating the posterior cervical muscles from the spines and laminæ and dividing the ligamentum nuchæ, inter-spinous and inter-laminar ligaments. The wound was closed by apposing the post cervical muscles over the damaged spine by interrupted catgut stitches. The rest of the wound was closed by continuous catgut. During the operation, 5 of the 15 animals showed transient paresis of the hind limbs for 1 to 4 hours.

Stage II.—This was done after 8 to 10 days of the first operation when the external wound was completely healed up. The animals were acutely flexed at the neck just caudal to the site of injury under *x*-ray control. In 3 out of 15 animals, there was complete paralysis of the hind limbs with paresis of the fore limbs, whereas in the rest paresis of only the hind limbs was produced. These animals were observed to lie on the side of the trunk with no effort to move. Feeding had to be helped. After 48 hours the limbs became spastic. Four to six days after operation the animals were observed to stand on their legs if supported. In another 8 to 10 days time the animals behaved normally. By this method it became possible to produce in animals post-traumatic complete or partial paralysis of the limbs with subsequent full functional recovery within 2 weeks' time. This condition is akin to the phenomenon of spinal concussion in human beings observed following injury of the cervical spine.

45. Hydrocephalus in infants and children, investigation of its etiology and treatment under Dr. R. Nigam at the Medical College, Nagpur.

This report embodies the work done during the period ending 30th September, 1957. The work consists of two parts.

(*a*) *Experimental production of hydrocephalus.*—Two common laboratory animals, the albino rat and the dog, have been selected for

this study. The technique consisted of injection of either a suspension of Indian ink or a living suspension of *mycobacterium tuberculosis*, as grown from the discharge of a person shown active disease. Intrathecal injections were given under light ether anæsthesia using a tuberculin syringe and a given size 16-gauge needle. Four groups of animals were employed.

Eleven animals died as a result of the injection. Six died two to three weeks after the injection. All these animals were examined post mortem. Both macroscopic and microscopic examination was done. Two animals showed definite hydrocephalus with dilatation of the ventricles deposition of the dye in the meninges, leucocytic reaction and in one case thrombosis of one of the venous sinuses. All the successfully injected animals showed evidence of meningeal irritation with neck retraction and skew deviation of the head. Attempts were made to visualise the ventricles in the albino rat with pyelocil, in the living, but this was a failure. Macroscopic and microscopic changes of the arachnoid granulations in various age groups have been under study.

(*b*) *Clinical study of 4 hydrocephalic children* —No obvious cause was forthcoming, viz. specific infection, prenatal trauma, toxæmias of pregnancy in the mother, post natal trauma, or meningitis. Most children were quite normal at birth and showed head enlargement a little later. The obstruction was of congenital origin.

Investigation by ventriculography, indigo carmine test, C.S.F. examination with manometry, and physical, chemical and microscopic features of the fluid

Case No.	*Age*	*Type of hydrocephalus*	C.S.F. *pressure*	*Treatment*
1	2 years	Internal	300 mm.	Ventriculo-peritoneal shunt
2	2 months	Internal	200 mm.	Died after ventricular puncture.
3	3 years	Communicating	300 mm.	Died after ventriculography meningitis.
4	2½ months	Internal	300 mm.	Ventriculo-peritoneal shunt.

Ventriculo peritoneal shunt is a simple operation and is comparatively safe in a child of poor risk The value of ventriculo-uretric shunt in such children is also being studied.

46. Clinical and experimental studies on keloids under Prof. K.K. Ghosh, Medical College Hospital, Calcutta.

Object of work.— (1) Why and how keloids occur in certain individuals with or without operative trauma.

(2) To establish the relationship between the diffusion of extra-cellular fluids with the formation and recurrence of keloids in the selected sites.

(3) To evaluate the different modes of treatment.

In the last 6 months, 43 cases of keloids were encountered of which only 22 had primary keloids. These patients (22 only) were subjected to investigations and different modes of treatment.

On completion of detailed history taking and clinical examination, the patients were subjected to (1) ultraviolet fluoroscopic examination for determination of the nature of vascularity and diffusion of extra-cellular fluid, and (2) microscopic examination of the biopsy material before the institution of treatment for noting the histological structural detail.

The patients were then subjected to the following modes of treatment.—

(1) Deep *x*-ray in combination with surgery—12 cases.

(2) Cortisone infiltration in combination with surgery—7 cases.

(3) Cortisone infiltration alone—2 cases.

(4) Surgical excision only—1 case.

After the completion of treatment the patients were again subjected to ultraviolet fluoroscopic examination and the excised keloid was subjected to microscopical examination for determining the changes produced as a result of treatment.

The patients are then being followed up.

Observations on the investigation and treatment

(1) *Ultraviolet fluoroscopic examination.*—Appearance of patchy fluorescent zones in the keloid mass and a denser fluoresene in the peri-keloidal zone in ultraviolet fluorscopic examination suggests excessive diffusion of extracellular fluid at these sites. It has been observed further that the selected site where keloid usually occurs, viz., the front of the chest, the outer and anterior aspect of shoulders and a broad zone on the midline of abdomen emit brighter fluorescence than the adjoining skin surfaces in susceptible individuals.

In 9 cases there were marked diminution in fluoresence both in the keloid as well as in the peri-keloidal zone after treatment with deep *x*-ray. In one the fluoresence was absent altogether and in the other the fluoresence was *unaltered.*

Of the two cases treated by cortisone infiltration alone one had definite diminution whereas the other showed hardly any change.

(2) *Histological pattern and the changes produced by treatment.*—Untreated keloid has, more or less, a constant structural pattern. The skin lining the keloid shows occasional areas of parakeratosis. The skin appendages are absent in the secondary keloids but are present though in lesser number in the primary ones The sub-epidermal zone shows the collagenous connective tissue layer where there are masses of spindle-shaped fibroblastic cells of various size and thickness, with abundant collagenous matrix. The immediate sub-epidermal zone shows loosely woven collagenous meterial Blood vessels consist of endothelium lined spaces and are more numerous in the peripheral part of the fibrous tissue.

There are occasional areas of lymphocytic infiltration.

Cases treated by deep x-ray exhibited the following changes.—Minute ulceration in the epidermis. Prickle cell layer of the epidermis contained enlarged cells with granular cytoplasm. In the sub-epidermal zone there was œdema and hyalimisation of fibrous tissue which extended deeper. The layer as a whole looked more fibrous than cellular. The nuclei of connective tissue showed fragmentation. The blood vessels were lined by swollen œdemamatous endothelium. Sections stained by Pickworth's method showed very few vessels in the tissues.

Cases treated by cortisone infiltration.—These showed occasional brick red collagen consisting of granular amorphous cytoplasm with scattered nuclear remnants. There were occasional hæmorrhagic areas with beginning of organization.

(3) *Treatment by different methods*—In the 1st group of the 12 cases treated by deep *x*-ray along with surgery none has come back with recurrence so far. All the patients had more or less relief from symptoms after the completion of pre-operative deep therapy. On an average, the cases had 1450 r in 6 days time both before and after the surgical treatment.

2nd group of the 7 cases treated by cortisone infiltration following surgical excision of the keloid, 1 has come back with complete recurrence within 2½ weeks of the completion of treatment. On an average 1½ c.c. of inj. cortilacetate (1 c.c. equivalent to 25 mg.) was infiltrated in this scar at weekly interval for 5 to 10 weeks. The others in this group are still symptom-free.

In the 3rd group, only cortisone infiltration was done at weekly interval with 1½ c.c. of inj. cortilacetate both in the keloidal as well as in the perikeloidal zone. Two patients have been subjected to this mode of treatment so far. In both there was partial relief from symptoms after the 3rd injection. In one, the regression of the paramedian [illegible] the 5th injection, whereas in [illegible] emarkable even at the end of [illegible]

In the 4th group, only surgical excision was carried out. Only case subjected to this line of treatment came back with recurrence after 2 weeks inspite of healing by primary intention.

47. Investigation of the economic feasibility of using all-plastic, expendable blood-collecting and blood-transfusion equipment in providing a blood transfusion service for a rural area in Orissa under Dr. David R. Bassett, Dr. Balabhadra Mahapatra and Dr. Dhirendranath Mund at Distt. Sambalpur, Orissa.

Plastic expendable blood transfusion equipment has many advantages over conventional glass and rubber equipment ; it is useful in all blood-banking operations. and especially in rural medical practice (e.g. Primary Health Centre), civilian and military disasters, and for specialized hematologic uses. If this equipment were available in India, it would be possible to give transfusions in rural areas, without requiring the equipment and staff time which is necessary to clean and sterilize conventional equipment. A field research cum demonstration project was set up to investigate the economic feasibility of using plastic expendable equipment in comparison with conventional equipment, plus some related aspects of blood transfusion work in rural areas.

Twenty-two transfusions were given to 15 patients between March 1956 and September 1957, using plastic expendable equipment. The average cost per transfusion was Rs. 12.55 (*plus* additional customs charges for importing equipment averaging Rs. 4.50). Some of the plastic components can be re-used for various hospital purposes, thus reducing the total cost per transfusion. An average of 78 minutes of one physician's time was required for each transfusion.

The estimated cost of conventional glass and rubber transfusion equipment prepared according to the standards established at Calcutta Blood Bank, is Rs. 8.40. It has not yet been possible to set up blood transfusion services at the Government Hospitals at Bargarh or elsewhere, so that further cost data regarding conventional equipment has not yet been obtained.

Inquiries were made on the status of manufacturing plastic medical equipment in India.

The incidence of Rh (D) negativity in the population of this area is 1.89 per cent (S.D.$\pm$0.24 per cent). A simple method of providing a hearted, lighted surface for Rh-testing is described.

Transfusions were given using proven group 0 blood without crossmatch. No adverse transfusion reactions occurred.

It is possible to establish a simple, portable blood transfusion service for Rs. 180. A report has been prepared describing experience at Barpali in giving transfusions under rural conditions. Eight Physicians have been trained in the practice of blood transfusion therapy.

VDRL slide test has been learned by 3 Barpali Village Service physicians, and demonstrated to 5 other physicians and 2 laboratory technicians. The technique is simple, reliable, and can be performed with simple and inexpensive equipment costing about Rs. 165. Three hundred and eighty-three tests were performed. Thirty-five specimens

were sent to the Antigen Production Unit Laboratory for checking results ; it appears that one has a tendency to over-read tests. However, there was no case in which we failed to detect a positive reaction which was reported positive by the Antigen Production Unit Laboratory.

48. Inquiry on development of biologically specific histochemical method for the localization of pituitary hormones under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

1. *In vitro* stimulation of corticoid synthesis under the influence of added ACTH has been studied It is felt that coupling of oxidative phosphorylation may be necessary for optimum synthesis of corticoids.

2. A new and specific method for the histochemical localization of corticoids has been developed, which consists of oxidising the alpha-ketol grouping of corticoids with $FeCl_3$ to an aldehyde compound Aldehyde compounds are then stained by leucofuchsin or Schiff's reagent.

3. Ovarian sections show evidence of $FeCl_3$—Schiff positive material. This observation is likely to give valuable information regarding the pituitary-adrenal-ovary relationship.

4 A new approach for increasing the ACTH secreting cells of the pituitary is being developed. For this the animals are adrenalectomized and the adrenals transplanted in the spleen. With this procedure the renal impairment is prevented and the adrenalectomized animals appear to survive apparantly without the addition of sodium chloride in drinking water.

49. Survey of malignant lesions of the ora-pharynx in the population of Guntur District and to evaluate pre-disposing and exciting causes, if any, f
under Dr. D.

Outpatients of the Government General Hospital, Guntur, were examined for the presence of oropharyngeal carcinoma for the period of August to December 1957. Sixty-six cases of epitheliomata were found in the oropharynx as against 65 epitheliomata registered for the same period in sites other than oropharynx. In the latter series carcinoma cervix is found to be 66 per cent of the total. Forty-two
males and
noma of the
opharyngeal
percentage
in the female. Equal incidence in both sexes but considerably less number was observed of carcinomata of the floor of the mouth, alveolar margin and the larynx. Four cases of the carcinoma palate were exclusively found in the females. One of these patients is in the habit of smoking the chutta keeping the burning end inside the mouth.

The association of smoking of chutta was significant in the majority of male patients with carcinoma cheek and tongue and they have been moderate to heavy smokers. In the female chewing of betel, with or without tobacco, was found to be of significant causal importance. In only a very minority of female patients history of smoking chutta was obtained.

In a small but negligible number, neither history of smoking nor of chewing could be elicited. History of addiction to alcohol was recorded in an occasional case or two and this bears no causal importance.

Leukoplakia was recognized in 2 cases. V. D. R. L. test was done as a routine in most of the cases and only in a small number it was positive.

Barring the isolated case of chutta, cancer observed in the female, the other three cases of palatal cancer noticed in the female are different, from that reported from Visakhapatnam.

In 25 per cent of the oropharyngeal carcinoma registered vitamin deficiency, by way of angular stomatitis, is noticed and in an equal number oral sepsis was also recognized. It is difficult to indicate whether oral sepsis preceded or is consequence of cancer.

In about a 3rd of the cases pigmentation of oropharynx was observed. This pigmentation was also noticed in the control groups in whom precancerous or cancerous lesions were not noticed but have given definite history of smoking chuttas. It is intended to carry out biopsy study of these pigmented spots with a view to recognize any pre-cancerous lesion as was suggested by Reddi and Rao (*Ind. Jour. Med. Sc., 11*. pp. 791-798, 1957.)

During this period inpatients admitted into the City Hospital for diseases other than oropharyngeal carcinoma and also staff of the institutions were examined for oropharyngeal carcinoma. In 3,000 cases so examined no malignant lesions of oropharynx was spotted in non-smokers, or smokers belonging to the age group 30 to 70.

Verification was also made for the incidence of oropharyngeal carcinoma in various communities resident in Guntur but no significant variation in incidence of the oropharyngeal carcinoma from community to community was observed and this is probably due to common habits of smoking and chewing in various communities resident in Guntur. Carcinoma tonsils and larynx appears to be much less in number in our series when compared to those reported by Khanolkar (*Acta Un. Int. Cancer*, p.6, 881, 1950). It is proposed to carry out the survey of age group 30 to 70 in the local factories and also in certain parts of Guntur municipality with the sole object of spotting the early malignant lesions of oropharynx and record available predisposing precipitating factors.

50. Studies on the physiology of dermatophytes under Dr A N Chakraborty at the School of Tropical Medicine, Calcutta.

During the period commencing from 11th May, 1957 to 20th September, 1957 the following lines of work were undertaken.—

I. *Culture of dermatophytes* —Scrapings from the scales of the skin were taken from 20 cases of ringworm infection out of which 12 proved to be positive for *T. rubrum* culturally. Of the remaining 8 cases, 3 did not show any growth and 5 were contaminated with *Aspergillus*, *Penicillium* and *Helminthosporium*, respectively.

II. *Cytochemical studies of the culture* —Normal cytochemical patterns of these fungi were studied. Some cytochemical entities (*e.g*, alkaline phosphatase, polysaccharides, calcium, iron and lipids) were studied at various ages of growth of the fungus grown on different culture media. It was seen that the reaction for alkaline phosphatase, polysaccharides and lipids were definitely more intense in the fungi grown on Sabouraud's glucose-agar media than those grown on other media (*e.g*, Sabouraud's maetose-agar, cornmeal-agar, nutrient agar, conservative media and agricultural media).

Localization c
standard as well as
gave better result.
was found between 3 and 6 hours.

Polysacch rides were found to be present mostly in outerwalls of the hyphæ and microconidia and in inter hyphal septa.

Presence of calcium, iron and lipids has been demonstrated for the first time in *T. rubrum* Here, too, both the techniques were employed of which the modified one proved satisfactory.

Iron, both in ferrous and ferric form, as well as in inorganic and organically bound form was observed in hyphæ and microconidia.

Lipids were demonstrated by Sudan black B and Sudan III technique. Culture done on media incorporated with these two dyes showed normal growth of the fungus and concentration of the dye within the hyphæ ond microconidia in the form of globules.

Inorganic salts of calcium was demonstrated by standard methods both in the hyphæ and microconidia as scattered grains in the cytoplasm and also in the nucleolus.

III. *Histochemical studies on the infected skin.*—Work has just been started on histochemical studies of the skin infected with ringworm and a few skin biopsy material have been collected from culturally proved cases.

HAEMATOLOGY

51. Hæmatological unit under Dr. J.B. Chatterjea at the School of Tropical Medicine, Calcutta.

I. Nutritional Macrocytic Anaemia

(*A*) *Assay of vitamin* B_{12} :

(*a*) *Serum vitamin* B_{12} *level.*—In a series of 25 uncomplicated cases, the mean values of free and total B_{12} level were 9.9 (range 0 to 82) and 96.5 (range 0 to 330) micro-microgram per ml., respectively. In 14 cases the total vitamin B_{12} level was lower than 90 micro-microgram per ml. These values are significantly lower than the corresponding values for normal Indians. After treatment with specific anti-megaloblastic drugs the level of total vitamin B_{12} definitely increased.

(*b*) *Vitamin* B_{12} *binding-capacity of serum.*—The vitamin B_{12} binding-capacity of serum was measured by estimating the serum level of free and total B_{12} following incubation *in vitro* of 1 ml. of serum with 1000 micro-microgram of B_{12} at 37°C for two hours. There was significant rise of free and combined fractions following such incubation.

(*B*) *Folic acid assay* :

(*a*) *In serum and urine.* –As reported last year, folic-acid levels in serum and urine vary widely and do not present any pattern significantly different from that in the control groups. It appears, therefore, that folic-acid levels in serum and urine *per se* provide no dependable guide to the existence of folic-acid deficiency.

(*b*) *Differential test of urinary folic-acid excretion after test dose of folic acid.*—Twenty-four hours' excretion of folic acid in urine was measured after administration in sequence of 5 mg. folic acid, (*i*) subcutaneously, and (*ii*) orally. In N.M.A., the mean excretion of folic acid after subcutaneous and oral test doses were 0.79 (range 0.19 to 1.38) and 0.45 (range 0.12 to 1.65) mg., respectively. After treatment, the mean excretion values after subcutaneous and oral test doses were 1.25 (range 0.12 to 2.3) and 0.78 (range 0.19 to 1.38) mg., respectively. With a similar technique, mean normal value after subcutaneous dose was 2.58 (range 1.45 to 3.72) mg. and after oral test dose was 1.83 (range 1.15 to 2.52) mg.

II. A Thalassaemic Syndrome

During this period comprehensive investigation was done in a series of 86 cases with a view to characterize them as fully as possible. It was found that in this series there were 74 instances of Hb.E-thalassæmia, 11 instances of homozygous thalassæmia and 1 instance of Hb.S-thalassæmia.

(*A*) *Comparative study of Hb.E-thalassæmia and homozygous thalassæmia.*—Comparative study in these two groups did not present any differentiating clinical and hæmatological features Homozygous thalassæmia *per se* did not appear to present any qualitative change significar
Clear-cut
through

(*B*) *Differential hæmoglobin pattern in Hb.E-thalassæmia disease.*—Relative proportion of the hæmoglobins E,F and A was measured in a Densitometer. Hb F was measured by alkali-denaturation technique. In a series of 20 cases, the mean values of Hb E,F and A were 67.1 per cent (range 45 to 83.5 per cent), 24 4 per cent (range 5.8 to 49 per cent) and 8.5 per cent (range 0 to 28.3 per cent) respectively.

These data corroborate the view that thalassæmia gene, while enhancing the expressivity of Hb.F, tends to suppress the formation of Hb.A. Expression of Hb.E is also disproportionately increased.

(*C*) *Family studies.*—In each of the 74 cases with Hb E-thalassæmia disease, Hb.E in the heterozygous form was always present in either parent. Percentage of Hb E in the heterozygous parent varied from 30 to 63.

(*D*) *Incidence of Hb E and thalassæmia heterozygote in general population.*—In a series of 700 unrelated Bengalee Hindus were encountered 2 instances of Hb.EE, 25 instances of AE and 26 instances of heterozygote thalassæmia (AA).

(*E*) *Comparative data on Hb.E heterozygote and thalassæmia heterozygote.*—Critical analysis of various data in the two groups showed that in general, abnormalities of erythrocytic morphology were more pronounced in thalassæmia heterozygote Owing presumably to the relatively higher percentage of Hb E in Hb.E heterozygote (AE) over Hb A_2 component in thalassæmia heterozygotes (AA), paper electrophoretograms in general clearly differentiated the two heterozygote conditions.

III. HAEMOPHILIA SYNDROME

In a series of 15 patients with evidence of deficient thromboplastin formation manifested by prolonged coagulation time and/or poor
f anti-hæmophilic globulin
'TC), and plasma thrombo-
. 3 and 1 respectively. In addition, in one male patient there was evidence of combined deficiency of AHG and PTC.

52. Inquiry on anæmias in infants and children under Professor H.I. Jhala at the Grant Medical College, Bombay.

For the purpose of this report the analysis is divided into two parts Part I deals with antenatal cases and their new-born. In the previous report ending September 1956, 73 cases were reported. Thirty-two cases were examined subsequently, and this work was concluded

after completing investigation of 105 cases. Part II contains the analysis of the results of investigations on anæmic infants and children. Last year 55 cases were reported. Since then 145 more children have been examined, bringing up the total to 200 cases. However, this report contains data on 150 cases as the remaining are being worked up.

Following are the total number of investigations done so far :—

Hæmatological	485	Serum Fe	206
Follow up	143	Serum B_{12}	208
Bone marrow	148	Sickling	311
Dietary	152	Fetal Hb	178
Rh grouping	220	Paper electrophoresis	13
Serum proteins	137	Families investigated	16

53. A survey of occurrence of sickle-cell trait and sickle-cell disease or its subtypes in Mahar Community of Nagpur and its neighbouring villages under Dr. R.N. Shukla at the Medical College, Nagpur.

(*A*) A survey was carried out amongst the adult labour population of the local textile mills, which employ them from different districts covering roughly an area of 100 miles radius surrounding Nagpur. The labourers belong to low economic group of the local population and predominently were from the Mahar, Kunbi, Maratha, Teli, Gond, Koshti and Mohameddan communities. In all 1,010 cases were examined. The presence of sickling was demonstrated by the method of Daland and Castle (1948). Fresh reducing solutions were made daily and the preparations were examined within two hours. All positive cases of the day were re-examined next day to eliminate false positive results. Peripheral smears were examined in all cases for morphological erythrocytic abnormalities and the presence of target cells. In cases showing the presence of target cells in peripheral smears and sickling by wet sealed preparation, hæmoglobin analysis was done by paper electrophoresis and alkalidenaturation technique for confirmation.

Results :

INCIDENCE OF SICKLE-CELL TRAIT IN CENTRAL INDIA.

Castes	Number of Persons examined	Number of persons positive for sickling	Percentage of incidence	Frequency of Sickle cell gene
Mahar	450	100	22.2	0.12
Kunbi	116	11	9.4	0.05
Teli	80	9	11.3	0.06
Gond	53	0	0.0	0.00
Koshti	46	0	0.0	0.00
Mohameddan	68	0	0.0	0.00
Brahmin	26	0	0.0	0.00
Miscellaneous	171	0	0.0	0.00
Total	1,010			

positive sickling cases showed the A S pattern, thus confirming the presence of sickle cell hæmoglobin in heterozygous form.

(B) Total number of cases of sickle-cell disease detected from this area so far were seven, out of which four were of sickle-cell anæmia and three of sickle-cell thalassemia. The diagnosis of these cases were confirmed after detailed investigation, hematological (including hæmoglobin analysis by paper electrophoresis and alkali-denaturation technique) and genetical.

Experimental studies on acquired hæmolytic anæmia under Dr. N.N. Sen at the Seth S. Karnani Memorial Hospital, Calcutta.

The work done was mainly concerned with the production of antisera in rabbits against guinea pig red cells and qualitative and quantitative study of these sera produced during different periods of immunization and by different methods of immunization.

Techniques for the detection of 'incomplete' antibodies have been standardized. Rabbits have been immunized against guinea-pig red cells by varying doses, by varying intervals and by different routes of injection. Titre of the antisera by different methods and at different periods have been determined. Effect of temperature on the titre thus obtained has been investigated.

55. A study in the pathogenesis of anæmia in infections under Dr. V. S. Mangalik at the K. G. Medical College, Lucknow.

In all 75 normal healthy individuals have been investigated These include medical graduates and students of whom 45 were males and 30 females. They varied from 65 kg. to 85 kg. in weight and in no case the hæmoglobin value was below 12 0 g. per cent and total serum proteins below 6.0 g. per cent.

1. TECHNIQUES EMPLOYED

(a) *Hæmatologic techniques.*—All estimations were carried out within four hours of the withdrawal of blood from the anticubital vein into wintrobe's double oxalate mixture. Red cells were enumerated in

Thoma's pipette in a dilution of 1 in 200, using isotonic sodium-chloride solution as the diluent. Two-pipette two-chamber method was employed as advocated by Ham and the standard error of the red cells count was determined in each case. Total leucocytes were counted in a dilution of 1 in 20, using 3 per cent acetic acid as the diluent. Hæmoglobin determinations were performed by acid hæmatin technique of Sahli. For the study of red cell morphology and differential leucocyte counts, peripheral smears were drawn on glass slides and stained with Wright's stain. Wintrobe's hæmatocrit was employed for the determination of packed red cell volume.

(b) *Biochemical techniques*: Plasma iron was determined by a modified technique of Hamilton. To 1.5 c.c. of plasma was added 0.75 c.c. of 2N hydrochloric acid and the mixture allowed to stand for 10 to 15 minutes after thorough shaking. 0.75 c.c. of trichlor-acetic acid was then added with vigorous stirring by a glass rod. This mixture was left for 20 to 30 minutes, and then centrifuged at 3,000 r.p.m. for 30 minutes. The supernatent was decanted off. To 1.8 c.c. aliquot of the supernatent were added the following in this order :

0.05 c.c. thioglycolic acid.

0.40 c.c. (0.1 g./100 c.c.) O-phenonthroline.

0.75 c.c. saturated sodium acetate.

The tube was shaken and allowed to remain at room temperature with a reagent blank (0.90 c.c. glass distilled water, 0.45 c.c. 2N HCl and 0.45 c.c. trichlor acetic acid *plus* reagents above) for 30 minutes. Optical density was measured in a Beckman's spectrophotometer model B at 510 mμ.

Total and unsaturated iron-binding capacity as also the per cent satuaration of iron in the plasma calculated as suggested by Cartwright and Wintrobe (*Jour. Clin. Invest.*, *28*, p. 46, 1949). For the determination of plasma copper and the copper content of the red blood cells and the whole blood, the techniques of Gubler *et al.* (*Jour. Biol. Chem.*, *196*, p. 209, 1952) were followed.

Glassware used in the collection, storage and estimation for iron and copper was carefully cleaned to make it copper-iron free by washing thoroughly with soap and water, rinsing, emmersing in potent potassium dichromate—sulphuric acid cleaning solution for one hour, and then rinsing with tap-water, simple distilled water, and finally with double distilled water, prepared in a all glass still. All the reagents used, including anti-coagulants were of A-R grade and of known iron-copper content. Matching of colour was performed on a Beckman spectrophotometer model B at 510 mμ for iron and 440 mμ for copper.